

COLLOID SYMPOSIUM MONOGRAPH

PAPERS PRESENTED AT THE SIXTH SYMPOSIUM
ON COLLOID CHEMISTRY, UNIVERSITY
OF TORONTO, JUNE, 1928

EDITED BY
HARRY BOYER WEISER
PROFESSOR OF CHEMISTRY, THE RICE INSTITUTE

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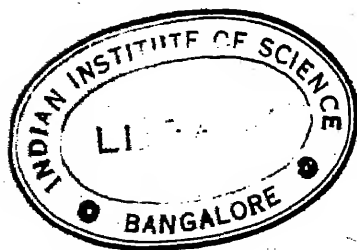
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FOREWORD

The Sixth Colloid Symposium, sponsored by the Colloid Division of the American Chemical Society and the Committee on the Chemistry of Colloids of the National Research Council, was held at the University of Toronto, Toronto, Ontario, Canada, June 14, 15 and 16, 1928. The international character of the symposium is indicated by the fact that one-third of the papers published in full in this volume were given by colloid scientists outside of the United States. Sir William B. Hardy of Cambridge, England, was the guest of honor.

On the closing day of the Symposium the two hundred members in attendance adopted unanimously the following resolutions:

"We, the members of the Sixth Colloid Symposium in convention assembled at Toronto, desire to express by this resolution appreciation to President Falconer and the Board of Governors of the University of Toronto for the excellent accommodations which have been afforded us and for the cordial welcome which we have received.

"We further desire to thank Professor Burton and the entire local committee on arrangements for the many efforts which have been expended on our behalf.

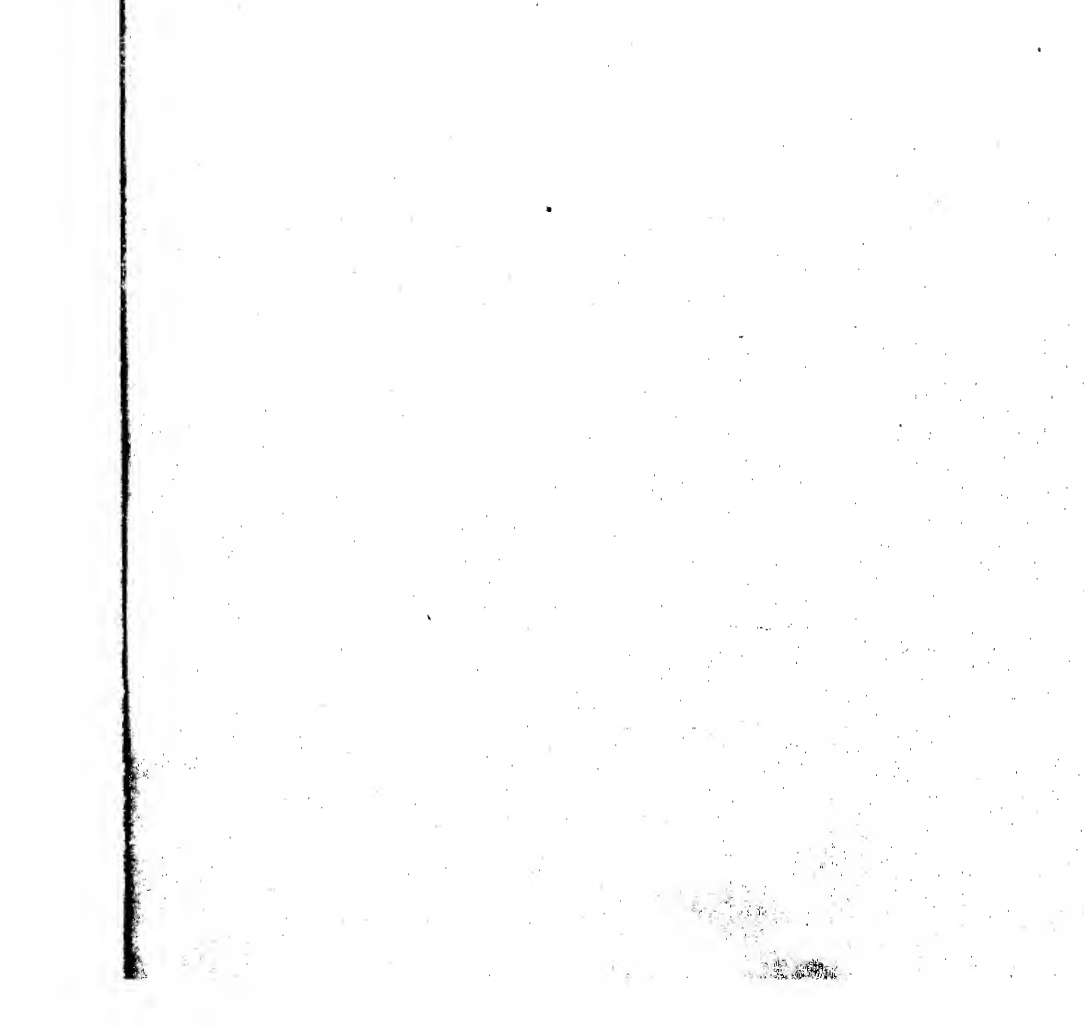
"Furthermore, we desire to express our pleasure and appreciation to Sir W. B. Hardy who has traveled far to honor us and the University of Toronto by accepting the post of Guest of Honor of this Symposium.

"We feel that this Symposium will be a bond between the various fields of natural science and an added evidence of the international good will which binds together the peoples of Canada and the United States."

The Seventh Symposium on Colloid Chemistry will be held at the Johns Hopkins University, Baltimore, Maryland, June 20, 21 and 22, 1929.

HARRY B. WEISER,

Houston, Texas.



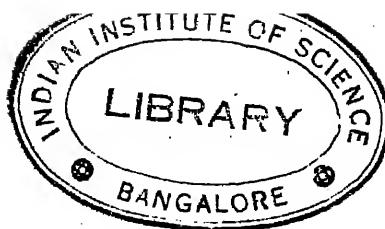


TABLE OF CONTENTS

Papers read at the Sixth Colloid Symposium held at the University of
Toronto, June 14, 15, 16, 1928.

	PAGE
1. LIVING MATTER—Sir William B. Hardy	7
2. ELECTRICAL RELATIONS AT SURFACES, THE SPREADING OF LIQUIDS, THE THICKNESS OF SURFACE FILMS AND THE DROP WEIGHT AND RING METHODS FOR THE DETERMINATION OF SURFACE TENSION—William D. Harkins	17
3. SURFACE CONDUCTANCE—David R. Briggs	41
4. THE EFFECT OF ADSORBED WATER ON THE ELECTRICAL CONDUCTIVITY OF POWDERS—F. B. Kendrick and F. J. Giffen	53
5. THE ACTIVITY AND ADSORPTION OF p-TOLUIDINE IN THE SURFACE OF ITS AQUEOUS SOLUTION—J. W. McBain, W. F. K. Wynne-Jones, and F. H. Pollard	57
6. ADSORPTION OF SODIUM OLEATE AT THE AIR-WATER INTERFACE—M. E. Laing, J. W. McBain, and E. W. Harrison	63
7. THE ADSORPTION OF METHYLENE BLUE BY LEAD SULFATE—Wilder D. Bancroft and C. E. Barnett	73
8. THE EFFECT OF TEMPERATURE ON THE COAGULATION OF COPPER COLLOIDAL SOLUTION—E. F. Burton and Beatrice Reid Deacon	77
9. THE STRUCTURE OF SOFTWOODS AS REVEALED BY DYNAMIC PHYSICAL METHODS—Alfred J. Stamm	83
10. FRACTIONATION OF DIPHTHERIA ANTITOXIC PLASMAS—P. J. Moloney and Edith M. Taylor	109
11. CATAPHORESIS OF BLOOD CELLS AND INERT PARTICLES IN SOLS AND GELS AND ITS BIOLOGICAL SIGNIFICANCE—Harold A. Abramson	115
12. METHODS OF STUDYING THE SURFACES OF LIVING CELLS WITH ESPECIAL REFERENCE TO THE RELATION BETWEEN THE SURFACE PROPERTIES AND THE PHAGOCYTOSIS OF BACTERIA—Stuart Mudd, Balduin Lucké, Morton McCutcheon and Max Strumia	131
13. THE RÔLE OF HEMOGLOBIN IN THE BLOOD—A. Baird Hastings	139
14. THE EFFECT OF EMULSIFICATION IN THE PEPTIC SYNTHESIS OF PROTEIN —H. Wasteneys and H. Borsook	155
15. EMULSIONS AND THE EFFECT OF HYDROGEN-ION CONCENTRATION ON THEIR STABILITY—John C. Krantz, Jr., and Neil E. Gordon	173

	PAGE
16. NEW MICROSCOPIC METHODS IN CONNECTION WITH THE PROBLEM OF VULCANIZATION—E. A. Hauser, H. Miedel and M. Hünemörder . . .	207
17. PREPARATION AND PROPERTIES OF AQUEOUS RUBBER DISPERSIONS—H. L. Trumbull	215
18. STUDIES OF ORGANOPHILIC COLLOIDS—G. S. Whitby, J. G. McNally and W. Gallay	225
19. THE INFLUENCE OF ELECTROLYTES AND NON-ELECTROLYTES UPON THE OPTICAL ACTIVITY AND RELATIVE RESISTANCE TO SHEAR OF GELATIN SYSTEMS—J. R. Fanselow	237
20. INFLUENCE OF GEL STRUCTURE UPON THE TECHNOLOGY OF SMOKELESS POWDER MANUFACTURE—Fred Olsen	253
21. GRAIN GROWTH IN SILVER HALIDE PRECIPITATES—S. E. Sheppard and R. H. Lambert	265
22. THE UNIFORM DISTRIBUTION OF CATALYSTS THROUGHOUT POROUS SOLIDS —Harry N. Holmes and Robert C. Williams	283
23. THE DEVELOPMENT OF THE ULTRACENTRIFUGE AND ITS FIELD OF RESEARCH—J. B. Nichols	287
24. THE STUDY OF HYDRATION CHANGES BY A VOLUME-CHANGE METHOD—H. A. Neville and H. C. Jones	309
25. ADSORPTION OF IONS AND THE PHYSICAL CHARACTER OF PRECIPITATES—Harry B. Weiser and G. E. Cunningham	319
AUTHOR INDEX	343
SUBJECT INDEX	345

COLLOID SYMPOSIUM MONOGRAPH

LIVING MATTER

BY SIR WILLIAM B. HARDY

My object when I began this address was to sketch the present position of what might be called the mechanistic theory of life, but I soon found that, if a theory be an inference from positive knowledge, there is no such theory. There is, it is true, a hypothesis, or in more direct phrase, a guess, and about it in the literature of the subject an accretion of analogies and scraps of physics and chemistry which, as biological tests are rarely applied to them, are probably irrelevant to the real issues.

The fact is, a biologist today is pretty much where an engineer would be if he knew even in detail the cycle of chemical changes which took place within an internal combustion engine but was wholly ignorant of the disposition of the moving parts.

I must not seem to belittle attainment. This century has seen immense advances in biology, but when one is trying to think in terms of mechanism they lie off the picture, and for two reasons, either because they are inexplicable, or because they are advances in our knowledge of the results of the activity of living matter and not of the working of the machine itself.

In my student days some forty years ago, there was a great body of knowledge of form and function, but living matter itself was accepted in a curious way as something inevitable and mysterious. The great third edition of Foster's Text Book of Physiology, which may fairly be said to have made both the British and American schools, contains only three pages of generalities concerning living matter. Strictly speaking it is silent as to the inner nature of protoplasm.

The mystery of life is as great now as it was then, and it were wise to recognize the fact.

My thesis soon narrowed itself down to the ungrateful task of dis-

playing the difficulties of the problem, and as a first step, let me state a belief which I have held for thirty years or more. It is that nothing is to be gained by claiming living matter as colloidal. There is as much reason to call it crystalloidal as colloidal and that much is just no reason at all. When a sufficient definition of colloid is forthcoming, it will be time seriously to consider whether living matter conforms to it. At present the colloidal kingdom seems to be an Alsatia wherein difficult states of matter find refuge from a too exacting enquiry.

A definition of the colloidal state which certainly had a vogue was in terms of the size of the units. An emulsion which turned up fortuitously in the course of some work on lubricants must be held to have disposed finally of a definition of that kind. It was a stable emulsion whose droplets were uniformly three millimeters in diameter. By shaking it violently the droplets could be broken up but, on standing, they reverted slowly to the standard size—three millimeters in diameter! By no test was it other than a typical solution save in its Gargantuan dimensions.

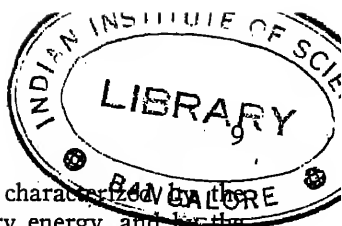
The most curiously life-like substance to my knowledge in the non-living world was a wax whose behavior was described by Kohlrausch more than fifty years ago. Obviously it was a plastic solid but there is no justification save an unwarranted extension of the term for calling it colloidal. Kohlrausch states that a cylinder of this wax hung from one end, then bent in succession say north, east, north, south and so on, would when left to itself, repeat the movements but in reverse order. Now the life-like quality lies not in the running down of a store of energy but in the existence of a timing device.

Looking back to my student days I seem to see an advance towards a knowledge of the mechanism of life in three directions. Of these, the first and most important is the accumulation of evidence which points to the existence of two processes distinguishable from one another and corresponding to the structural division into nucleus and cell substance. The chemical cycle of the former involves nitrogen and it is concerned with growth and repair. It is doubtful whether this process can cease without involving the living cell in that irreversible change called death. The chemical cycle does not include the production of simple acids such as lactic acid.

The other process is characteristic of the cell substance, that is to say, of the portion of the living cell which envelops the nucleus and which is immediately responsive to those variations in the environment which the physiologist calls stimuli.

This process—I use the singular though it may vary in character from mass movement to the specialized molecular wave known as the nervous impulse, or the movement of water and complex substances

LIVING MATTER



in the act of secretion—this process, I say, is characterized by the oxidation of carbohydrate to produce the necessary energy, and by the production of acid. There is also a change of electrical potential, the material in the active state being galvanometrically negative to that in the resting state.

The best known example, the one that has been most fully worked out, is the contraction of skeletal muscle. The normal cycle includes two phases, an active phase which is anaerobic and in which lactic acid appears, and an aerobic phase where glucose is oxidized and the energy set free utilised in part to restore the "loaded" state. This normal cycle, however, is conformed to only when the fuel glucose and oxygen are present in adequate quantities. With the elusiveness characteristic of living matter, wide departure from the normal cycle occurs when either fuel or oxygen is deficient.

I have ventured to include the act of secretion of a gland cell, such as a cell of the kidney or salivary gland, in this class though I believe there is still doubt as to whether this cell involves either a variation of electrical potential or the appearance of acid. With respect to the former, I find it difficult to believe that water-holding electrolytes can be drawn through even living matter without there being a potential gradient. It is certain that the normal act of secretion involves the expenditure of energy for reasons familiar to every physiologist, the chief being the fact that it is accompanied by an uptake of oxygen.

The measurable phenomena of an organ are the sum of a very large number of changes. They are statistical phenomena and difficulty in detecting an electromotive change may be due merely to lack of orientation of the units.

The proof of the existence of two distinguishable fundamental processes in the living cell can hardly be summarised, it is so abundant and diverse. There is, first of all, the many experiments ranging from cutting operations on unicellular animals, such as *Stentor*, or on the nerve cell, to the analysis of the properties of the ovum.

A most profound observation was made by Chambers. When the nucleus is injured, repair is impossible, the entire cell perishes, and there is no production of acid. But when the cell substance is injured, repair and recovery of state commonly follow and at the seat of injury acid is formed.

Set this last fact alongside the other well-known fact that the death change in skeletal muscle consists in, amongst other things, a catastrophic destruction of the carbohydrate reserve and the appearance of lactic acid.

If the detection in a general way in the working of the machine of two fundamental processes is the first great advance, the second as-

surely is the attainment of some degree of certainty as to the nature of the moving parts.

It will be well here to admit complete ignorance of the nucleus machine. We do not even know that it is a machine in the proper sense of the word. Are there cyclic changes of energy in which the free energy is directed to particular purposes? If anyone has the answer to this question, I must confess ignorance of it.

But, despite this ignorance, there are two orders of facts which finally fix upon proteins as the moving parts, no matter what the processes may be. The first is the certainty that the diversity in the machine which determines the differences between the strains of living matter on the earth—between the hundreds of thousands of plants and animals—is based not upon their chemically active parts, such as their engines, but upon differences in the chemical constitution in the proteins out of which the living substance is mainly built up. The processes of digestion and such phenomena as anaphylaxis and the precipitin reactions prove this.

The second line of evidence is furnished by what we know of muscular contraction. The moving parts of any machine are those parts which direct the available energy and are not themselves implicated directly in the chemical cycle. To take the second character first,—it is certain that proteins, despite the fact that they form some eighty per cent of the solids, are not directly involved in the chemical cycle of contraction of muscle. A muscle is an internal combustion engine which normally burns carbohydrate.

This assurance as to the significance of proteins is a great advance on the position in my student days when their chemistry was only guessed at, and when they partook of the mystery of life itself. I recollect receiving reproof from one of my elders when I attempted a straightforward explanation of the behavior of certain proteins in solution.

The third great advance is in the recognition of ferments as the active agents in the machine. In my early days ferments were still classified as organised and unorganised. The former were living cells, such as yeast cells, the latter free ferments produced by living cells. I cannot recall anyone bold enough to suggest that inside the cell were ferments whose business it was to control chemical change.

Buchner made the first great necessary step when he conducted alcoholic fermentation in the absence of living cells but by ferments extracted from living cells. Since then endoferments have been demonstrated in plenty. Meyerhoff, for example, has prepared from muscle an extract practically free from protein which contains a catalyst, or

catalysts, which reproduces *in vitro* the changes in carbohydrate characteristic of intact muscle.

Let us look a little more closely at this question of catalysts, for it is not without its difficulties. Clearly their activities are controlled during life because at death they become a disorderly mob who pull the very fabric to pieces, protein and all becoming involved in the breakdown. They are during life rigidly restrained, subject to a co-ordinating timing device in the intact machine.

It is a plausible suggestion, due to Jordan Lloyd, that the proteins exert the control. There are plenty of instances in the chemistry of living matter. Haemoglobin, the red pigment of blood, carries oxygen from the air in the lungs to the tissues, and the dissociation constants of the haemoglobin of different animals are such as fit it for the peculiar needs of that animal. Now haemoglobin is composed of an active molecule haematin which, though capable of oxidation and reduction, is totally unsuited to carry oxygen in the blood. It is only when combined with protein that it is fitted for its task. And there is more. Haematin seems to be the same in all animals in which it is found, but the protein ally is different. The inference is so obvious that it need not be stressed.

A few lines earlier I said that at death the catalysts escape from control. Why? Because the structure of the moving parts gets disorganized, but structure here is not gross but molecular structure. Let us look into this for it leads to a curious problem.

At the death of an animal, its muscles die sooner or later, apparently because in the absence of oxygen it cannot work and to maintain itself, it needs must expend energy. It is true that the firing mechanism can be put out of gear without general destruction. Foster and Fearon, for example, found that frog's muscles at 0° and in abundant oxygen became non-irritable, but they were not dead for recovery took place when the temperature was raised. There was not even carbohydrate destruction and production of lactic acid *provided the tension of oxygen was high enough*.

When oxygen is withheld and death occurs, the proteins are hydrolyzed and the change can be followed quantitatively by estimation of the soluble nitrogen. In about four days a steady state is reached, but further breakdown can be started by freezing the muscle. And now the catalysts have finally escaped from control—hydrolysis shows no signs of stopping even in sixteen days.

This effect of freezing and thawing has been ascribed to mechanical injury produced by the ice crystals. This is not likely since a typical degradation or coagulation of the proteins takes place when the expressed and filtered juice from the muscles is frozen and thawed.

This kind of change is common in colloidal solutions. Milk, a solution of chlorophyll-like muscle juice, can be coagulated by freezing and thawing, but the molecular changes need time for their accomplishment and therefore, as is well known, they can be avoided if both cooling and thawing are sufficiently rapid. I do not recall a colloidal solution which does not give this evidence of a time lag in the process of coagulation.

Now here are two facts which seem to me to have immense significance. By no procedure has it been found possible to freeze and thaw living muscle without causing instant death. Whatever the key structure may be, the presence of what means life, there is no evidence of a time lag in its destruction.

The second fact is that it is not cold which destroys, but dehydration. The freezing point of frog's muscle is -0.42° , and muscle can be frozen and thawed without causing death unless a critical temperature is overpassed, namely -2.0° . The quantity of water withdrawn in the form of ice at -2° can be calculated. It is 77.5 per cent of the total water and Moran, to whom these facts are due, finds that 77.5 per cent of the water can be removed from living frog's muscle by simply drying over calcium chloride without killing it. The muscle when the water is restored is again irritable and has the alkaline reaction and other qualities of living muscle. Muscle is not killed by cold. It can be overcooled to -4° for days without impairment of its properties.

That the living machine is in part, at any rate, a surface energy machine is an hypothesis due, I believe, in the first instance to the physicist FitzGerald. The only case in which it has been possible vigorously to examine the hypothesis has broken down. A. V. Hill finds that the surface tension needed within a muscle fibre is 4800 dynes—an impossible figure. Mine's careful and indeed brilliant work furnishes the best evidence I know of, that the living machine is a surface energy machine, but after all what does the immense literature on the influence of electrolytes amount to? No more than this—that electrolytes like the electric current and other agents, mechanical and chemical, can act as stimuli to alter the rate of working. It is not even certain that the presence of electrolytes is necessary for the maintenance of the machine though, if present, a due balance of concentration is necessary. I hasten to say that the firing mechanism seems to depend for its working upon the presence of electrolytes.

For the maintenance of the machine, four cations are needed, those of calcium, magnesium, potassium, and sodium, and in certain relative concentrations. We do not at all know how they act, though the work of Chambers seems to suggest that they control the internal viscosity and therefore probably electrical potential differences. The chapter on

electrolytes may almost be said at present to begin and end with Macallum's profound observation that the necessary electrolytes are those of sea water where living matter probably had its origin, and the necessary relative concentrations also are those of sea water.

The muscle fibre is, so far as its proper function movement is concerned, an internal combustion engine working within singularly narrow limits of temperature. The thermodynamic problems raised by this, I have not space to deal with. Since it works only within narrow limits of alkalinity and is subservient mainly to cations it must, I suppose, be accorded the general characters of an electro negative colloid.

The only exception which, so far as I know, can be taken to the statement that the structure of the machine can be maintained only by continued expenditure of energy is the case of drying mentioned above. It is not possible to conceive of normal chemical change continuing in a muscle dried to the consistency of a tough glass.

The movement of certain forms of marine amoebæ has been analysed by Pantin. Each individual is a minute cylinder and it progresses straight forward in the direction of the major axis. The more external part or wall of the cylinder is viscid, the internal part more fluid, and movement is due to the internal fluid flowing forwards and bursting through to the exterior when it at once assumes the consistency of the wall. In the meantime, the wall at the hind end becomes more liquid and flows in.

This simple example of movement is worth mentioning because it is an instance of the adaptation of a wide-spread and possibly universal property of living matter to a simple end. The property is the increase in viscosity which accompanies the active state.

It is well to remember that this simple animal does much more than move. It is sensitive to changes in its surroundings, it selects its food, it grows and multiplies, preserving its type.

Growth and reproduction are perhaps the most abiding characteristics of living matter. Neither is to be confused with the growth of a crystal for it is a dynamic state which increases in volume and reproduces itself.

Keeping in mind the unit of life, the cell, we see at once that the capacity is limited. A whale and a flea are complexes of cells and the individual cell is of much the same size in both.

Broadly, it may be said that when a unit of living matter grows to a certain size it becomes unstable and divides, producing two daughter units. But the limiting size varies from that of the submicroscopic, filter passing virus of many diseases to those curious crawling plants, the Myxomycetes, in which the volume of an individual may amount to a cubic centimeter.

There does, however, appear to be a fundamental relation, for these larger cells always have more than one nucleus. It looks as though the volume ratio of cell substance to nuclear substance cannot exceed a certain value.

When we bear in mind Chamber's observation on the influence of the nucleus upon repair, it seems safe to conclude that the limiting factor is the distance at which the nucleus ceases to be able to exert its control. The range of influence of the nucleus estimated in this way is of the order of the range of the capillary attraction of a solid surface in orienting the molecules and so controlling the structure of a fluid with which it is in contact.

Here apparently is a secure conservative conclusion, yet, with characteristic elusiveness, living matter furnishes an exception so stupendous as to be derisory.

A nerve fibre consists essentially of a delicate cylinder about three millimeters in diameter. This cylinder, called the axon, is the very tenuous outgrowth of a nerve cell. It may, in a large animal, be meters in length, yet the capacity for growth and repair of every part of this process is wholly dependent on the nerve cell from which it has its origin—that is, upon a microscopic speck of matter only fifty to a hundred μ in its major axis. Let the axon be cut at no matter what the distance from the nerve cell and the part cut off dies and disintegrates, whilst the part which retains connection with the nerve cell not only persists but, if opportunity offers, it will ultimately grow down to remake contact and restore function.

This fact is a commonplace of the text book of physiology. To me it has always been almost the most intriguing mystery of living matter. How shall the capacity for gathering unformed and unlike matter to itself persist in so specialized a structure as the axon of a nerve fibre? And how shall the capacity be controlled by a portion of matter of negligible size situated a yard away?

Any theory of the mechanism of life must take account of its amazing elusiveness. Considered from the standpoint of purpose, we may say that it can become as labile as it needs or as obstinately persistent. Of its fidelity to type, heredity offers countless examples. Of its labile qualities, let this instance suffice:

In the course of his remarkable experiments on newt embryos, Sheeman has found that most tissues transplanted at an early age from one situation to another adopt the character of the new environment. Potential skin, for example, transplanted to brain becomes brain, whilst potential brain transplanted to skin becomes skin. When we contrast the qualities of skin, a mere integument, and of brain, the seat of the most mysterious aspects of life, this mutual convertibility becomes

simply fantastic. It is even possible to overpass the limits of species. A portion of potential skin of species *A* planted amid the potential brain of species *B* becomes brain, but it remains a portion of the brain of *A* habited within the brain of *B*!

Let us in conclusion consider the ovum as a physical system. Its potentialities are prodigious and one's first impulse is to expect that such vast potentialities would find expression in complexity of structure. What do we find? The substance is clouded with particles but these can be centrifuged away, leaving it optically structureless but still capable of development.

On the surface of the egg there is a fine membrane, below it fluid of high viscosity, next fluid of relatively low viscosity, and within this, the nucleus which is, in the resting stage, merely a bag of fluid enclosed in a delicate membrane. How shall sources and sinks of energy be maintained in a fluid composed, as to over 0 per cent, of water? They undoubtedly are there for the egg is a going concern, taking in oxygen and maintaining itself by expenditure of energy.

Clearly the ovum is possible only as a paradox. It is no pangenetic structure—a mosaic of all the parts to which it will give origin. Tristram Shandy's theory is false. To play its part it can only be the simplest form of living matter, but its simplicity is neither that of a machine nor of a crystal but of a nebula. Gathered into it are units relatively simple but capable by their combinations of forming a vast number of dynamical systems into which they fall as the distribution of energy varies. After all, a nebula holds within itself the beginnings of a history more complex even than that of an ovum and yet, so far as structure is concerned, it is but a simple affair!

The more there is known about living matter the more there is revealed a curious simplicity. Sheeman finds skin transmuted to brain or brain to skin, but the agent which effects the change appears to be a chemical substance probably of quite ordinary character. You may lead living matter as you may a donkey with a carrot—but you have to choose the carrot with some care.

Biology halts on the mechanical side because it needs the services of men who are at once real physicists and real biologists—both faculties being within the same brain. Biochemistry has made its great advances because it has been served of late by men who are both real chemists and real biologists.

Our difficulties need restatement by a physicist with ingrained knowledge and wide experience of the properties of matter. I can perhaps illustrate my meaning by a phenomenon of great biological importance, namely chemotaxis. When microscopic free-swimming organisms are present in water of uniform quality, they distribute themselves at ran-

dom; but if a gradient of chemical reaction be established by the introduction of a trace of weak acid into one part of the water, they gather in the more acid or less acid region according to their nature and previous history. The biologist has been accustomed to express facts such as these in terms of purpose, as an attraction towards or repulsion from the acid.

The facts are, however, capable of expression in much more concrete terms. Let us assume that the acid slows the rate of movement of the organisms; what will be their final distribution? Kinetic theory tells us that they will gather where their velocity is least.

If, as I firmly believe, biology stands now in urgent need of real physicists, how shall such men gifted with biological insight be obtained? Most readily by making a new form or crystallization center of knowledge.

All of you will recollect that curious intellectual ferment which led in the seventeenth century to the foundation in Italy, France and Great Britain of so many academies devoted to learning. It was preceded and in part accompanied by the promulgation of schemes, often fantastic but always interesting, for "Colleges," all of which bore the stigmata of their derivation from the monastic ideal. Each scheme included a set of rules which should govern conduct.

Let me give you my ideal of a Biological College. It should have three floors—a ground floor for molecular physics, a first floor for biophysics, and a top floor for cell mechanics. And of the staff the professor of molecular physics should have no responsibility for biology, the professor of biophysics should be a Mr. Facing-both-ways, responsible to physics and to biology, whilst the professor of cell mechanics should be a biologist pure and simple. That college I should expect to provide the new synthesis of knowledge of which biology stands in need.

Of rules there should be none and of precepts only one—To publish is good but not to publish is better. I would that precept could have been followed with respect to this address!

Cambridge, England.

ELECTRICAL RELATIONS AT SURFACES, THE SPREADING OF LIQUIDS, THE THICKNESS OF SURFACE FILMS, AND THE DROP WEIGHT AND RING METHODS FOR THE DETERMINATION OF SURFACE TENSION

By WILLIAM D. HARKINS

INTRODUCTION

The electrical relations at interfaces, the spreading of liquids on liquids and solids, and the structure and thickness of surface films, are of fundamental importance in connection with colloidal phenomena. The purpose of this paper is to present a few observations concerning each of these topics.

The drop weight and ring methods for the determination of surface tension are so widely used that it seems of importance to add an account of work done for the purpose of correcting some of the incorrect ideas still prevalent concerning them.

I. ELECTRICAL RELATIONS AT SURFACES

It is well known that small particles which are suspended in an electrically conducting medium are given a definite mean of velocity (v) by an impressed electrical field, and that this velocity is very nearly a linear function of the strength of the field (X). The theory of this phenomenon, known as cataphoresis, and more especially of the related electrical endosmosis has been developed by Helmholtz,¹ Lamb² and von Smoluchowski.³

The theory is represented by the two following equations:

$$\frac{v}{X} = \frac{1}{4\pi} \frac{D(\phi_2 - \phi_1)}{\eta} \quad \text{Helmholtz [1]}$$

$$\frac{v}{X} = \frac{1}{4\pi} \frac{D(\phi_2 - \phi_1)}{\eta} \frac{l}{d} \quad \text{Lamb [2]}$$

$$D(\phi_2 - \phi_1) = \frac{4\pi\eta}{X} \quad \text{[3]}$$

¹ *Wied. Ann.*, 7, 337 (1879).

² *Phil. Mag.*, 25, 52 (1888).

³ *Bull. Int. de L'Academie des Sciences de Cracovie*, 1903, p. 182.

Here D is the dielectric constant, and η is the viscosity of the double layer. Since both D and η are unknown, it is not possible to obtain the value of the potential difference ($\phi_a - \phi_i$) of the double layer.

However, if equation [1] is written

$$\frac{V}{X} = \frac{1}{4\pi} \frac{D}{\eta} \zeta \quad [4]$$

in which D and η are the respective values for the outer phase (commonly an aqueous solution), then zeta (ζ) is a fictitious potential whose value may be calculated.

As a special case, an emulsion of hexane produced in a 0.1 molar solution of sodium oleate may be considered. When equal parts of the oil and the aqueous solution are mixed by a motor-driven egg beater, it is found that the droplets of hexane vary in diameter from less than 0.2μ to about 10μ , with the largest number at about 1 to 1.5μ .

Determinations of the amount of soap adsorbed⁴ indicate that each droplet of oil in a stable emulsion is surrounded by a monomolecular film of soap—possibly in some cases by a somewhat thicker film. Since soap is partly hydrolyzed, the film may be supposed to consist of molecules of both sodium oleate and oleic acid. The total number of negative oleate ions and oleic acid molecules in the film around a droplet of 1μ diameter is of the order of 10,000,000 or 15,000,000. Consider that the hydrocarbon portion of the oleate ions is oriented toward the hexane, and the COO^- group toward the water. Assume that the sodium (Na^+) ions are held in close juxtaposition by the electrostatic forces between the unlike charges. The oil droplet, from this point of view, is merely a polyvalent ion of very high charge.

While oleate ions undergo diffusion between the surface layer and the aqueous phase, the number of such ions per unit area in the surface is very great, so that, with Helmholtz, this may be considered as one plate of a condenser. This may be supposed to possess only molecular irregularities. The distribution of the positive ions may be considered to be determined by the velocities of diffusion and the electrostatic attractions (and repulsions) in accord with some unknown distribution function.

Thus each droplet is surrounded by a diffuse,⁵ but very thin, layer of positive ions. If no current is passed the distribution may be considered as spherically symmetric provided the droplets are sufficiently far apart.

If a current is passed through the solution the symmetry of the ionic

⁴ Griffin, *J. Am. Chem. Soc.*, **45**, 1648 (1923); Van der Meulen and Rieman, *ibid.*, **46**, 876 (1924); Harkins and Beeman, "Colloid Symposium Monograph," Vol. 5. The Chemical Catalog Co., Inc., New York, 1928, p. 31.

⁵ Gouy, *J. Phys.* (4), **9**, 452 (1920).

atmosphere is affected and in such a direction as to decrease the velocity which the negatively charged droplet would otherwise have in a direction toward the positive electrode. The positive sodium ions move in a direction which is on the whole toward the negative electrode, but on account of the attraction of the negatively charged surface of the drop, on the whole they follow its curvature. These positive ions (with a relatively minute number of negative ions) carry the solvent with them, not at the same, but at a smaller velocity. The movements are conditioned by the viscosity of the medium, which may be supposed to be more or less influenced by the proximity of the surface, the more particularly since this has a high density of electrical charge. The dielectric constant is also affected by the same factors.

If the droplet of hexane rises under the influence of gravity, Stokes' law may be expected to hold. If the droplet is moved by the application of an electric field, its velocity need not follow this law on account of the electrical flow of solvent around it. However, it seems to be worth while to see how the electrical potential difference obtained by the direct application of Stokes' law compares with the fictitious ζ potential calculated on the basis of incorrect assumptions from the equations of Helmholtz or of Lamb.

It is found that the velocity of such a particle corresponds, according to Stokes' law, to a negative charge of 2.340 on a particle 1μ in diameter, *i.e.*, if all of the 10,000,000 or 15,000,000 molecules of the soap except the 2,430 were completely un-ionized, and if the 2,430 molecules of sodium oleate were so completely ionized that the 2,430 Na^+ ions are at an infinite distance, then the oil droplet should have a negative charge equal to that of 2,430 univalent negative ions. The equation is

$$N = \frac{6\pi r \eta v}{e X}$$

in which e is the charge on the electron, N is the number of charges, η is the viscosity of the solution, and v/X is the mobility or the velocity for unit potential gradient. The potential ^a of a charged sphere is

$$\phi = \frac{Ne}{Dr} \quad \text{so} \quad \phi = \frac{3\pi\eta v}{D X}; \quad \frac{v}{X} = \frac{1}{6\pi\eta} \frac{D}{r} \phi$$

According to this equation, the potential ϕ is 84 millivolts for the droplet in question. The apparent potential ϕ obtained by the use of the equation of Stokes is always 3/2 the fictitious zeta (ζ) potential usually given in books on colloid chemistry.

According to the relations given above: (1) The effective ionization

^a The use of this apparent potential instead of the fictitious zeta potential was suggested to the writer by Professor A. C. Lunn.

(N) of a colloidal particle varies directly as its radius. (2) The effective ionization per unit area (N/A) varies inversely as the radius of the particle, and therefore directly as the curvature of the surface. (3) The apparent potential ϕ for the particle is independent of the radius. Therefore the potential is the same for all spherical particles of the same material in any certain solution. (4) The effective ionization (N) and the apparent potential (ϕ) depend upon the nature of the particle and upon the nature of the medium in which it is suspended. From the foregoing it is apparent that the curvature of the surface determines the extent of the apparent ionization at the surface; the greater the curvature the greater the ionization.

It is of interest that the apparent potential (ϕ), obtained so simply above, has exactly the same value as the potential (ψ) of the particle against the liquid as given by the theory of Debye and Hückel for spherical particles.

It is commonly assumed that the large and small droplets exhibit the same velocity in an electric field. It has been shown by Mooney, however, that the larger droplets move the faster, but that the difference is much smaller in the presence of electrolytes than with pure water. Thus any of the apparent potentials (ϕ , ζ , ψ) is somewhat larger for a large than for a small droplet.

II. FILMS: THE SPREADING OF LIQUIDS AND THE FINAL SPREADING COEFFICIENT

(WITH BERNARD GINSBERG)

INTRODUCTION

The spreading of liquids over other liquids or over solids is a phenomenon of great importance. The surface of water or of any solid in nature and in the laboratory is almost universally a contaminated and not a pure surface, and very elaborate methods are often essential if most of the film is to be removed or kept from forming. The film on an impure or contaminated surface may be formed by spreading from a liquid or a solid surface, or it may arise through a condensation from the vapor, as will be illustrated later in this paper.

The conditions which determine the spreading or non-spreading of a liquid were treated more than fifty years ago by Neumann,¹ who considered the triangle of *forces* which acts at one edge of a lens. Since, however, some investigators appeared to doubt the general applicability of such a relation, the criteria were expressed by Harkins² in a form

¹ "Theorie der Capillarität," Leipzig, 1894 (from Lectures given in 1861-73).

² Harkins and Feldman, *J. Am. Chem. Soc.*, 44, 2665 (1922).

which involves only *thermodynamic magnitudes*. The most suitable thermodynamic potential function for this purpose appeared to be the ζ -function of Gibbs, commonly designated as the Gibbs free energy (F).

THERMODYNAMICS OF THE SPREADING COEFFICIENT⁹

The maximum work (δW) done on a surface when its area (σ) is increased by $d\sigma$ is

$$\delta W = -\gamma d\sigma + p dv \quad [1]$$

The value of the free energy of Gibbs is defined by the equation

$$F = U - ST + pv \quad [2]$$

and the Helmholtz free energy by

$$A = U - ST \quad [3]$$

It follows that

$$F = A + pv \quad [4]$$

and

$$dF = dA + p dv + v dp \quad [5]$$

But, since

$$dA = -dW - SdT - Tds \quad [6]$$

$$dF = -dW + p dv + v dp \quad [7]$$

From [7] and [1]

$$dF = \gamma d\sigma + v dp - SdT - Tds \quad [8]$$

so that at constant pressure and temperature

$$\left(\frac{\partial F}{\partial \sigma}\right)_{p,T} = \gamma \quad [9]$$

and

$$\left(\frac{\partial F}{\partial \sigma}\right)_{p,T} d\sigma = \gamma d\sigma \quad [10]$$

For a *saturated surface* γ is a function of p and T only, so

$$\Delta F = \gamma \Delta \sigma \quad [11]$$

and for unit area, $p, T = \text{constant}$

$$\Delta F = \gamma \quad [12]$$

The condition that a liquid b shall by itself spread on a liquid a , at constant temperature, is thus that the summation of the terms $(\partial F / \partial \sigma)_{p,T}$ is negative, that is less than zero. Thus, if b spreads on a ,

⁹ This development was not given by Harkins and Feldman.

the surface of *a* disappears and, for a sufficiently thin layer, an equal area of the surface of *b* and of the interface *ab* appear. The increase of free energy is

$$dF = \left(\frac{\partial F}{\partial \sigma_b} \right) d\sigma_b + \left(\frac{\partial F}{\partial \sigma_{ab}} \right) d\sigma_{ab} + \left(\frac{\partial F}{\partial \sigma_a} \right) d\sigma_a \\ = \gamma_b d\sigma_b + \gamma_{ab} d\sigma_{ab} + \gamma_a d\sigma_a \quad [13]$$

For a film of appreciable thickness the effects of gravitation must also be considered; but they may be neglected in the limiting cases treated in this paper.

The condition for spreading to occur starting from any given condition, is

$$dF < 0 \quad [14]$$

and for non-spreading

$$dF > 0 \quad [15]$$

If it is assumed that

$$d\sigma_b = d\sigma_{ab} = -d\sigma_a \quad [16]$$

which is practically valid for a very thin layer, then

$$\frac{dF}{d\sigma} = \gamma_b + \gamma_{ab} - \gamma_a \quad [17]$$

Let $-dF/d\sigma$ be designated as the spreading coefficient (S') then

$$S'_b = \gamma'_a - \gamma'_b - \gamma'_{ab} \quad (p = \text{constant, the surfaces are saturated}) \quad [18]$$

Now if a drop of the pure liquid *b* is put on the surface of the pure liquid *a*, and these surfaces are considered before they become contaminated, then the initial coefficient of spreading S is given by the equation.¹⁰

$$S_b = \gamma_a - \gamma_b - \gamma_{ab} \quad [19]$$

After the liquids, the films, and the vapor attain equilibrium, the final coefficient of spreading (S'_b) is represented by the equation

$$S'_b = \gamma'_a - \gamma'_b - \gamma'_{ab} \quad [20]$$

THE SPREADING OF BENZENE ON THE SURFACE OF WATER

The interesting phenomena related to equations [19] and [20] may be illustrated by the spreading of benzene on water. Hardy states that

¹⁰ The initial value of the interfacial tension is not determined in practice, but it is commonly assumed that there is no great difference between its value and that of the final coefficient, which is what is determined.

benzene spreads on the surface of water, while Langmuir considers that it does not spread. The phenomena involved have been investigated by Harkins and Jordan.¹¹ They find that if enough pure benzene is dropped on the surface of pure water to give a layer about 1 mm. thick it spreads over the whole surface to give an apparently uniform layer. This behavior agrees with the fact that the initial spreading coefficient has a value of 8.9. As the benzene evaporates holes appear in the layer, and the optical effects indicate that the angle of contact is not zero. If the surface tension of the water in one of these "holes" is determined, it is found that here, where the film of benzene appears to be absent, it is actually present. That is, the water is covered with a monomolecular, or thicker, invisible film of benzene. The layer of benzene around the holes is sufficiently thick to be easily visible. The fact that this layer does not spread over the contaminated surface of the water in the hole is in accord with the value of the *final* spreading coefficient, which is found to be -1.49 ergs per sq. cm. and indicates the existence of a considerable angle of contact.

Before considering the general application of the final spreading coefficient it is advisable to discuss an empirical relation given by Antonow.

THE FINAL SPREADING COEFFICIENT AND THE ANTONOW RELATION

A general relation concerning interfaces, *which would be of extreme importance if it were true*, was stated by Antonow¹² in 1907 as follows: the interfacial tension between two liquids, mutually saturated with each other, is equal or very approximately equal to the difference between the surface tension of the two phases, each in contact with the vapor of the other phase.

The need for a careful consideration of this relation arises from the fact that practically all books on surface tension, or capillary chemistry, consider it to be valid.

If the Antonow relation were correct, then for mutually saturated liquids, interfaces and vapors

$$\gamma'_{ab} = \gamma'_a - \gamma'_b \quad [21]$$

in which γ'_a is larger than γ'_b .

Equation [20] for the final spreading coefficient of liquid *b* on *a* may be written:

$$S'_b = (\gamma'_a - \gamma'_b) - \gamma'_{ab} \quad [20']$$

If the Antonow relation were to hold, then the spreading coefficient

¹¹ Thesis, University of Chicago, 1927.

¹² *J. chim. phys.*, 5, 372 (1907).

of b on a would be zero, and that of a on b would be twice the interfacial tension, as may be seen from equation [22].

$$S'_a = -(\gamma'_a - \gamma'_b) - \gamma'_{ab} \quad [22]$$

Thus the Antonow relation expressed in terms of the final spreading coefficients becomes:

$$S'_b = 0 \quad [23],$$

$$S'_a = 2\gamma'_{ab} \quad [24]$$

Relation [24] is certainly an extremely peculiar one. *If the relation were true the phenomena of the spreading of liquids on liquids would be restricted in an extremely remarkable way.* Under equilibrium conditions no lenses of b could exist on the surface of the liquid a but lenses of a would always form on b . This is contrary to the experience of those who have investigated the phenomena. For example the initial spreading coefficient of n -octyl alcohol b on water a is at 20° C. and 1 atmosphere equal to -35.7 , so octyl alcohol spreads over an uncontaminated water surface with extreme readiness. Nevertheless, unless the amount of the alcohol per sq. cm. of surface is exceedingly small, lenses of the alcohol remain on the surface under equilibrium conditions, which shows that the final spreading coefficient is negative. This indicates that the difference between the initial and final coefficients of spreading is very large, and greater than 35.7 .

The difference of sign of the initial and final coefficients is quite general in case the initial coefficient is positive, but not if it is negative.

If a is water and b an organic liquid, then it is common, but not universal, that the final value of the surface tension of the aqueous phase is greatly lowered by the organic liquid, while the effect on the surface tension of the organic liquid is relatively small. Thus the final spreading coefficient of the organic liquid on water is lowered greatly, while the final coefficient for the spreading of water on the organic liquid is greatly increased. The approach to equilibrium conditions decreases the tendency of the organic liquid to spread on water, and increases the tendency of the water to spread on the organic liquid. This discussion does not apply to such organic liquids as the higher saturated paraffins, which have, when pure, little effect on the surface tension of water.

Antonow investigated six pairs of liquids and found his relation to be reasonably exact. The later more extensive work of Reynolds seems to indicate an even closer agreement with the rule.

The purpose of the experiments described in the present paper was to obtain more accurate data on a few pairs of liquids, and these data show very plainly that the relation of Antonow is very far from true. Thus the values (Table 1) of the final spreading coefficient of organic

TABLE I. *The Final and Initial Spreading Coefficients of Organic Liquids with Water at 20°. Experimental Data and Results.*

Organic Liquid	Benzene C_6H_6	Carbon Disulfide CS_2	Methylene Iodide CH_2I_2	n-Heptyl Alcohol $C_7H_{15}OH$	Isoamyl Alcohol $(CH_3)_2CH(CH_2)_3OH$
Oil water					
Density	0.99816	0.99862	0.99891	0.99796	0.99407
Radius of tip34385	.34385	.2902	.2902	.2902
Drop weight082624	.09379	.082525	.03187	.02906
Surface tension	62.36	70.49	72.20	28.53	25.92
Wet organic liquid					
Density87665	1.26315	3.31843 ^a	.83027 ^a	.82764 ^a
Radius of tip2900	.2900	.1887	.2902	.2902
Drop weight032106	.03570	.036604	.02952	.026325
Surface tension	28.82	31.81	50.52	26.48	23.56
Surface tension for dry organic liquid	28.86 ^b	31.38 ^b	50.68	26.97	23.73
Interfacial tension					
Radius of tip5980	.1887	.2761	.2761
Drop volume4311	.01440	.05071	.03186
Interfacial tension	35.03 ^b	48.63	45.87 ^c	7.95	5.00
Interfacial tension according to Antonow rule	33.54	38.68	21.68	2.05	2.36
Per cent deviation * from Antonow rule	4.44	25.72	111.58	287.8	111.86
Spreading coefficients					
Oil on water					
Initial	8.86	— 7.26	— 23.80	37.83	44.02
Final	— 1.49	— 9.95	— 24.19	— 5.90	— 2.64
Water on oil					
Initial	— 78.92	— 90.00	— 67.94	— 53.73	— 54.02
Final	— 68.75	— 87.31	— 67.55	— 10.00	— 7.36

^a The density of the dry methylene iodide was 3.32152; the density of the dry heptyl alcohol was 0.82209; the density of the dry isoamyl alcohol was 0.81038.

^b Harkins, Clark, and Roberts, *J. Am. Chem. Soc.*, 42, 700 (1920).

^c The value published by Harkins and Feldman [*J. Am. Chem. Soc.*, 44, 2665 (1922)] is 48.50. The value 45.87 given in this paper was checked carefully, but no error could be found in it.

* That is, the percentage of the interfacial tension as calculated by the Antonow rule, which must be added on in order to obtain the actual interfacial tension.

TABLE 2. *Initial Spreading Coefficients for Organic Liquids with Water.*

(Data mostly by Harkins, Clark, and Roberts.)

Surface tension of water = 72.75 dynes $t = 20^\circ$ $\gamma_o =$ Surface tension of organic liquid $\gamma_i =$ Interfacial tension $S_o =$ Initial spreading coefficient of oil on water $S_w =$ Initial spreading coefficient of water on oil				
	γ_o	γ_i	S_o	S_w
<i>I. Paraffins</i>				
Isopentane, $(CH_3)_2CHCH_2CH_3$	13.72	49.64	9.39	-108.67
Hexane, C_6H_{14}	18.43	51.25	3.07	-105.57
Octane, C_8H_{18}	21.77	50.81	0.17	-101.79
Di-iso-amyl (Decane), $((CH_3)_2CH(CH_2)_2)_2$	22.24	46.80	3.71	-97.31
"Stanolax"	30.69	55.55	-13.49	-97.61
Liquid petrolatum, Squibb.....	31.12	55.32	-13.69	-96.95
<i>II. Unsaturated Paraffins</i>				
Trimethyl ethylene, $\begin{matrix} CH_3 \\ >C:C< \\ CH_3 \end{matrix} \begin{matrix} H \\ \\ CH_3 \end{matrix}$	17.26	36.69	18.80	-92.18
Heptene, $CH_3(CH_2)_4C\equiv CH$	22.32	28.15	22.28	-78.58
<i>III. Primary Alcohols</i>				
Methyl, CH_3OH	22.61	0	50.14	-50.14
Ethyl, C_2H_5OH	23.14	0	49.61	-49.61
Propyl, C_3H_7OH	23.80	0	48.95	-48.95
Isobutyl, $(CH_3)_2CHCH_2OH$	22.8	1.80	48.15	-51.75
Isoamyl, $(CH_3)_2CH(CH_2)_2OH$	23.73	5.00	44.02	-54.02
Heptyl, $C_7H_{15}OH$	26.97	7.95	37.83	-53.73
Octyl, $C_8H_{17}OH$	27.53	8.52	36.70	-53.74
<i>IV. Secondary Alcohols</i>				
Methylhexylcarbinol, $CH_3CHOH(CH_2)_5CH_3$	26.52	9.61	36.62	-55.84
<i>V. Sulfur Alcohols and Sulfur Derivatives</i>				
Mercaptan, C_6H_5SH	21.82	26.12	24.81	-77.05
Carbon disulfide, CS_2	31.38	48.63	-7.26	-90.00
<i>VI. Ethers</i>				
β,β' -Dichloroethyl sulfide, $ClC_2H_4SC_2H_4Cl$	42.82	28.36	1.57	-58.29
Ethyl ether, $C_2H_5OC_2H_5$	17.10	10.70	44.95	-66.35
<i>VII. Aldehydes</i>				
Heptaldehyde, $CH_3(CH_2)_5CHO$	26.88	13.74	32.13	-59.61
<i>VIII. Ketones</i>				
Methyl ketone, CH_3COCH_3	24.15	6.28	42.32	-54.88
Methylbutyl ketone, $CH_3COCH_2CH_2CH_2CH_3$	25.49	9.73	37.53	-56.99
Methylhexyl ketone, $CH_3CO(CH_2)_4CH_3$	26.79	14.09	31.87	-60.05
Ethylpropyl ketone, $CH_3CH_2CO(CH_2)_2CH_3$	25.39	13.58	33.78	-60.94

	γ_0	γ_1	$\frac{S_0}{w}$	$\frac{S_w}{o}$
<i>IX. Chloroketones</i>				
Monochloro-acetone, $\text{CH}_3\text{ClCOCH}_3$	35.27	7.11	30.37	-44.59
Asym-dichloro-acetone, $\text{CHCl}_2\text{COCH}_3$	31.91	14.43	26.41	-55.27

X. Acids

Formic, HCOOH	37.6	0	35.15	-35.15
Acetic, CH_3COOH	27.63	0	45.12	-45.12
Propionic, $\text{C}_2\text{H}_5\text{COOH}$	26.7	0	46.05	-46.05
Butyric, $\text{C}_3\text{H}_7\text{COOH}$	26.8	0	45.95	-45.95
Isovaleric, $(\text{CH}_3)_2\text{CHCH}_2\text{COOH}$	25.33	2.73	44.69	-50.15
Heptylic, $\text{CH}_3(\text{CH}_2)_5\text{COOH}$	28.31	6.56	37.88	-51.00

XI. Unsaturated Acids

Undecylenic ($25^\circ\text{C}.$), $\text{CH}_3\text{C}_6\text{H}_9\text{C}_7\text{H}_{13}\text{COOH}$	30.64	10.14	31.97	-52.25
Oleic, $\text{C}_{17}\text{H}_{33}\text{COOH}$	32.50	15.68	24.57	-55.93

$\begin{array}{c} \text{—O—} \\ | \\ \text{—C=O Group} \end{array}$

XII. Esters Containing the

Isoamylbutyrate, $(\text{CH}_3)_2\text{CHCOO}(\text{CH}_2)_4\text{CH}(\text{CH}_3)_2$	25.19	23.00	24.56	-70.56
Ethyl isovalerate, $(\text{CH}_3)_2\text{CHCH}_2\text{COOC}_2\text{H}_5$	23.68	18.39	30.68	-67.46
Ethyl caproate, $\text{CH}_3(\text{CH}_2)_4\text{COOC}_2\text{H}_5$	25.81	25.46	21.48	-72.40
Ethyl nonylate, $\text{CH}_3(\text{CH}_2)_7\text{COOC}_2\text{H}_5$	28.04	23.88	20.83	-68.59

$\begin{array}{c} \text{—O—} \\ | \\ \text{—O—C=O Group} \end{array}$

XIII. Esters Containing the

Ethyl carbonate, $(\text{C}_2\text{H}_5\text{O})_2\text{C=O}$	26.31	12.86	33.58	-59.30
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XIV. Halogen Derivatives

Methylene chloride, CH_2Cl_2	26.52	28.31	17.92	-74.54
Methylene iodide, CH_2I_2	50.68	45.87	-23.80	-67.94
Chloroform, CHCl_3	27.13	32.63	12.99	-78.25
Bromoform, CHBr_3	41.53	40.85	-9.63	-72.07
Carbon tetrachloride, CCl_4	26.66	43.26	2.83	-89.35
Ethyl bromide, $\text{C}_2\text{H}_5\text{Br}$	24.16	31.20	17.39	-79.79
Ethylene dibromide, $\text{CH}_2\text{BrCH}_2\text{Br}$	38.71	36.54	-2.50	-70.58
Acetylene tetrabromide, $\text{CHBr}_2\text{CHBr}_2$	49.67	38.82	-15.74	-61.90
Isobutylchloride, $(\text{CH}_3)_2\text{CHCH}_2\text{Cl}$	21.94	24.43	26.38	-75.24
Ter. butylchloride, $(\text{CH}_3)_3\text{CCl}$	19.59	23.75	29.41	-76.91
Isoamyl chloride, $(\text{CH}_3)_2\text{CH}(\text{CH}_2)_2\text{Cl}$	23.48	15.44	33.83	-64.71
Perchloroethylene, C_2Cl_4	31.74	47.48	-6.47	-88.49
Tribromohydrin, $\text{CH}_2\text{BrCHBrCH}_2\text{Br}$	45.36	38.50	-11.11	-65.89

XV. Nitro Compounds and Nitrates

Nitromethane, CH_3NO_2	36.82	9.66	26.27	-45.59
Isoamyl nitrate,* $(\text{CH}_3)_2\text{CH}(\text{CH}_2)_2\text{NO}_2$..	(27.18)	(30.80)	14.77	-76.37

* The interfacial tension of isoamyl nitrate was not determined against pure water, but against a 0.177N KCl solution, since the liquid and pure water have almost identical densities.

TABLE 2. Initial Spreading Coefficients for Organic Liquids with Water.—
(Continued)

	γ_o	γ_i	S_o w	S_w o
<i>XVI. Nitrils</i>				
Acetonitril, CH_3CN	29.30	0	43.45	-43.45
Butyronitril, $\text{CH}_3(\text{CH}_2)_2\text{CN}$	28.06	10.38	34.31	-55.07
Isovaleronitril, $(\text{CH}_3)_2\text{CHCH}_2\text{CN}$	26.03	14.14	32.58	-60.86
<i>XVII. Amines</i>				
Dipropylamine, $(\text{C}_3\text{H}_7)_2\text{NH}$	22.54	1.66	48.55	-51.87
Di-isobutylamine, $((\text{CH}_3)_2\text{CHCH}_2)_2\text{NH}$..	22.05	10.28	40.42	-60.98
<i>XVIII. Aromatic Hydrocarbons</i>				
Benzene, C_6H_6	28.86	35.03	8.86	-78.92
Toluene, $\text{C}_6\text{H}_5\text{CH}_3$	29.89	36.06	6.80	-78.92
o-Xylene, $\text{C}_6\text{H}_4(\text{CH}_3)_2$	29.89	36.06	6.80	-78.92
m-Xylene, $\text{C}_6\text{H}_4(\text{CH}_3)_2$	28.72	37.89	6.14	-81.92
p-Xylene, $\text{C}_6\text{H}_4(\text{CH}_3)_2$	28.33	37.77	6.65	-82.19
Ethyl benzene, mesitylene, $\text{C}_6\text{H}_3(\text{CH}_3)_3$	28.51	38.70	5.54	-82.94
p-Cymene, $\text{CH}_3\text{-C}_6\text{H}_4\text{-CH}(\text{CH}_3)_2$	28.09	34.61	10.05	-79.27
<i>XIX. Halogen Derivatives</i>				
Chlorobenzene, $\text{C}_6\text{H}_5\text{Cl}$	33.08	37.41	2.26	-77.08
Bromobenzene, $\text{C}_6\text{H}_5\text{Br}$	36.26	39.83	-3.34	-76.32
Iodobenzene, $\text{C}_6\text{H}_5\text{I}$	39.70	41.84	-8.79	-74.89
o-Monobromotoluene, $\text{C}_6\text{H}_4\text{CH}_3\text{Br}$	35.85	41.15	-4.25	-78.05
α -Monochloronaphthalene, $\text{C}_{10}\text{H}_7\text{Cl}$	41.80	40.74	-9.79	-71.69
α -Monobromonaphthalene, $\text{C}_{10}\text{H}_7\text{Br}$	44.59	42.07	-13.91	-70.23
<i>XX. Amino Derivatives</i>				
Aniline, $\text{C}_6\text{H}_5\text{NH}_2$	42.58	5.77	24.40	-35.94
<i>XXI. Nitro Derivatives</i>				
Nitrobenzene, $\text{C}_6\text{H}_5\text{NO}_2$	43.38	25.66	3.71	-55.03
o-Nitrotoluene, $\text{C}_6\text{H}_4\text{CH}_3\text{NO}_2$	41.46	27.19	4.10	-58.48
m-Nitrotoluene, $\text{C}_6\text{H}_4\text{CH}_3\text{NO}_2$	40.99	27.68	4.08	-59.44
<i>XXII. Aldehyde Derivatives</i>				
Benzaldehyde, $\text{C}_6\text{H}_5\text{CHO}$	40.04	15.51	17.20	-48.22
<i>XXIII. Ethers</i>				
Anisol, $\text{C}_6\text{H}_5\text{OCH}_3$	35.22	25.82	11.71	-63.35
Phenetol, $\text{C}_6\text{H}_5\text{OC}_2\text{H}_5$	32.74	29.40	10.61	-69.41
<i>XXIV. Isothiocyanates</i>				
Phenyl mustard oil, $\text{C}_6\text{H}_5\text{NCS}$	41.44	39.04	-7.73	-70.35

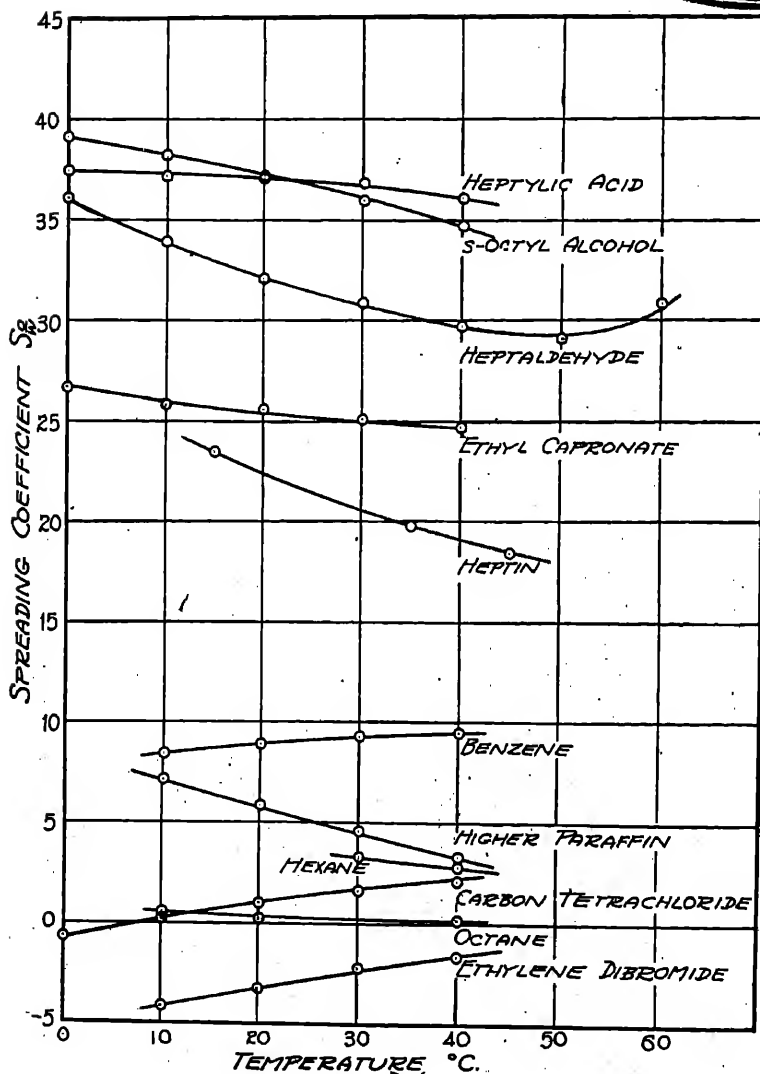


FIG. 1.—Initial Spreading Coefficients for Organic Liquids on Water at Various Temperatures.



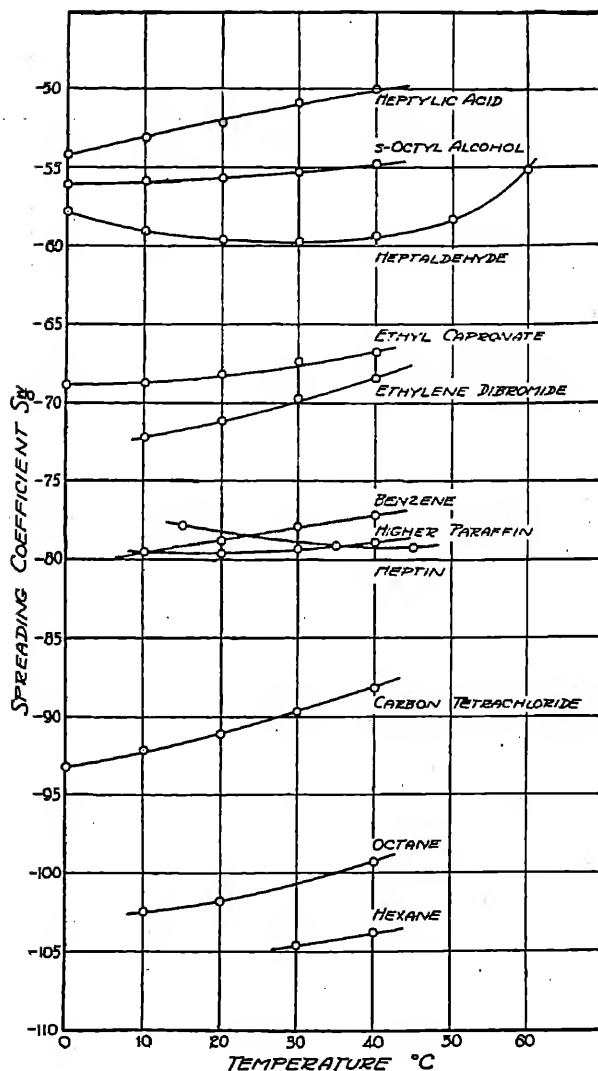


FIG. 2.—Initial Spreading Coefficients for Water on Organic Liquids at Various Temperatures.

TABLE 3. *Data for the Temperature Coefficients of Initial Spreading Coefficients.*
(Data by Harkins and Cheng.)

	$S_o =$ spreading coefficient for oil on water $S_w =$ spreading coefficient for water on oil				
	γ_o	γ_l	γ_w	S_o	S_w
Higher paraffin					
10°	30.87	36.16	74.22	7.19	-79.51
20°	30.04	36.87	72.75	5.84	-79.58
30°	29.20	37.42	71.18	4.56	-79.40
40°	28.45	37.82	69.56	3.29	-78.93
Benzene					
10°	30.26	35.56	74.22	8.40	-79.52
20°	28.90	34.96	72.75	8.89	-78.81
30°	27.61	34.34	71.18	9.23	-77.91
40°	26.25	33.84	69.56	9.47	-77.15
Heptin					
15°	22.80	27.15	73.49	23.54	-77.84
35°	20.88	29.66	70.38	19.84	-79.16
45°	19.88	30.38	68.74	18.48	-79.24
s-Octyl alcohol					
0°	27.93	8.44	75.64	39.27	-56.15
10°	27.17	8.80	74.22	38.25	-55.85
20°	26.28	9.24	72.75	37.23	-55.71
30°	25.51	9.65	71.18	36.02	-55.32
40°	24.74	10.04	69.56	34.78	-54.86
Heptaldehyde					
0°	28.64	10.78	75.64	36.22	-57.78
10°	27.72	12.51	74.22	33.99	-59.01
20°	26.84	13.74	72.75	32.17	-59.65
30°	25.84	14.41	71.18	30.93	-59.75
40°	24.96	14.82	69.56	29.78	-59.42
50°	24.08	14.50	67.91	29.33	-58.33
60°	23.19	12.13	66.18	30.86	-55.12
Heptylic acid					
0°	29.84	8.34	75.64	37.46	-54.14
10°	29.05	7.93	74.22	37.24	-53.10
20°	28.14	7.54	72.75	37.07	-52.15
30°	27.39	7.13	71.18	36.66	-50.92
40°	26.49	7.00	69.56	36.07	-50.07
Ethyl capronate					
0°	27.89	21.03	75.64	26.72	-68.78
10°	26.88	21.42	74.22	25.92	-68.76
20°	25.87	21.29	72.75	25.59	-68.17
30°	24.90	21.15	71.18	25.13	-67.43
40°	23.81	21.02	69.56	24.73	-66.77
Carbon tetrachloride					
0°	29.38	46.97	75.64	-.71	-93.23
10°	28.05	45.97	74.22	.20	-92.14
20°	26.70	45.05	72.75	1.00	-91.10
30°	25.54	44.04	71.18	1.60	-89.68
40°	24.41	43.04	69.56	2.11	-88.19

TABLE 3. *Data for the Temperature Coefficients of Initial Spreading Coefficients.—*
(Continued)

	γ_a	γ_D	γ_w	$\frac{S_o}{w}$	$\frac{S_w}{o}$
Ethylene dibromide					
10°	40.28	38.28	74.22	-4.34	-72.22
20°	38.79	37.20	72.75	-3.24	-71.16
30°	37.54	36.08	71.18	-2.44	-69.72
40°	36.15	35.03	69.56	-1.62	-68.44
Octane					
10°	22.73	51.01	74.22	.48	-102.50
20°	21.77	50.81	72.75	.17	-101.79
40°	19.82	49.58	69.56	.16	-99.32
Hexane					
30°	17.22	50.66	71.18	3.30	-104.62
40°	16.27	50.48	69.56	2.81	-103.77

liquids on water is found to be -1.49 for benzene, -9.95 for carbon disulfide, -24.19 for methylene iodide and -5.90 for heptyl alcohol, while according to Antonow's rule they should all be zero.

It may be noted that in all of these cases the final coefficient of spreading is lower than the initial coefficient and is negative in sign. The greatest lowering of the coefficient from initial to final is that for heptyl alcohol, which is lowered from 37.83 to -5.90.

The fact that the initial coefficient for a liquid of the type of *n*-octyl alcohol ($S = 35.74$) or heptylic acid ($S = 37.12$), is converted into a negative value in the final coefficient, shows that such great decreases are not uncommon.

It is to be expected that liquid pairs which give a positive initial spreading coefficient will not in general give such large magnitudes of the negative final coefficient as those which are already negative for initial conditions. From this point of view the relation of Antonow should hold much more closely for the spreading of the liquid *b* on the liquid *a* if the value of the initial spreading coefficient S'_b is positive.

It was believed at one time that the initial coefficient of spreading is universally positive, but Harkins and Feldman showed that it is negative in many cases for the spreading of an organic liquid on water and almost universally negative for the spreading of water on an organic liquid. If *b* is water or an organic liquid the initial coefficient for spreading on mercury *a* is positive in all known cases, while for the spreading of mercury on water or on an organic liquid it is negative.

Thus if the rule is to be used as a guide in predicting magnitude of an interfacial tension, as is common in books on surface tension and surface energy, its use should be restricted to the cases in which one of the final coefficients of spreading is positive, and the prediction

should not be expected to be at all exact, unless allowance is made for the negative value of S_b' , which is supposed by the rule to be equal to zero.

WORK OF ADHESION AND OF COHESION

The work of adhesion W_A' between two liquids at equilibrium is given by the relation

$$W_A' = \gamma_a' + \gamma_b' - \gamma_{ab}' \quad [25]$$

and the work of cohesion for the liquid b saturated with a is

$$W_{c(b)} = 2\gamma_b' \quad [26]$$

so

$$S_b' = W_A' - W_{c(b)}' \quad [27]$$

and

$$S_a' = W_A' - W_{c(a)} \quad [28]$$

If Antonow's relation holds, then $S_b' = 0$, and the work of adhesion between mutually saturated aqueous and organic phases is equal to the work of cohesion for the organic phase. However, the peculiar nature of the relation is made apparent by the fact that it predicts that the work of adhesion is not equal to the work of cohesion in the other (aqueous) phase. In general the rule predicts that the work of adhesion between any two mutually saturated liquid phases is equal to the work of cohesion in that phase which exhibits the smaller cohesion, but not equal to that in the other phase.

DETERMINATION OF THE SURFACE AND INTERFACIAL TENSION OF MUTUALLY SATURATED PHASES

The surface tensions were determined by the drop weight method, and the calculations were made by the use of the corrections of Harkins and Brown as given in International Critical Tables (Vol. IV). The organic liquid was shaken with water until equilibrium was attained. The drops are formed from the phase whose surface tension is desired. They are dropped into a special form of container (Fig. 3) with two ground glass joints. A portion of the other phase is held in the upper part of this container, the lower part of which is a weighing bottle into which the drops fall. It is important that the drop shall not be pulled over by suction, since this may disturb the equilibrium with the vapor. It is also important that the drop hang in the saturated vapor at almost full size until equilibrium is attained. For this purpose a method of

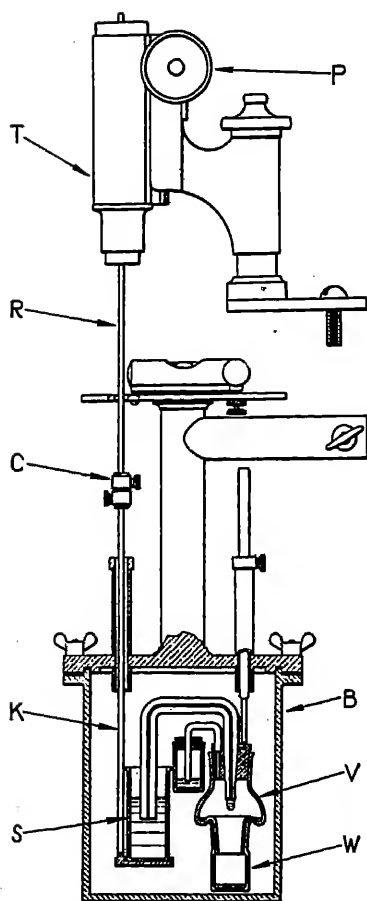


FIG. 3.—Apparatus for the Accurate Determination of Surface Tension by the Drop Weight Method.

- T—Bar moved by ratchet and pinion.
 R—Metal rod fastened to T.
 C—Metal connection for fastening R to K.
 K—Metal support for S.
 S—Supply bottle.
 P—Pinion head for adjustment of T.
 B—Nickel plated brass box.
 V—Glass container for liquid to saturate vapor phase.
 W—Weighing bottle for collecting drops.

control of the drop devised by H. N. Harkins, P. L. K. Gross, and W. D. Harkins was used. The supply bottle *S* which contains the phase under investigation is made adjustable in height. To secure good adjustment a stand with a ratchet and pinion¹³ is used. The supply bottle is held by a metal support *K* which is fastened to a metal rod *R* by means of a connection piece *C*. The rod *R* is fastened to the movable bar *T*. By turning *P*, a pinion wheel, *T* is raised or lowered and the height of *S* thus regulated. The supply bottle *S* is raised to start the formation of the drop and is then lowered. Next it is adjusted to such a height as to give the largest possible stable drop. The bottom of the largest stable drop is located by the position of the cross hair of a short focus telescope which has been carefully adjusted by an earlier trial. The period during which the drops were suspended at full extension was varied from three to six minutes per drop, without any perceptible variation in the weight of the drop which falls. For less soluble substances of low vapor pressure this time of suspension needs to be greatly lengthened.

In some cases, depending upon the vapor pressure and other factors, probably chiefly upon the difference between the temperature of the room and that of the thermostat, it was found necessary to cool *V*, which contains the liquid for saturating the vapor phase, before adjusting the apparatus in the thermo-

¹³ A microscope stand may be used.

stat, in order to prevent distillation into W while the desired temperature was being reached.

For the determination of the drop weight of a very volatile liquid such as carbon disulfide, it was found necessary to devise a special type of vent tube which is shown in Figure 3. Instead of having a vent hole through the brass tube and stopper (to which V is attached), a brass tube was soldered to the stopper around a small vent hole. The other end of the brass tube passed through another stopper provided with a vent hole. This second stopper supported a small weighing bottle containing just enough carbon disulfide so that its surface stands slightly above the outlet of the brass tube. In this way the loss by vaporization from the bottle in which the drops are being collected is almost entirely eliminated, and more constant drop weight results can be obtained than otherwise.¹⁴ A photograph of the nickel-plated box B in which the drop weight apparatus is contained has been published by Harkins and Brown.¹⁵ The box and the stand which carries the ratchet and pinion are clamped to separate iron stands in order that the drops may not be shaken by the adjustment of the height of the bottle S . The procedure for using the apparatus is very similar to that described by Harkins and Brown with changes due to the fact that for the present work suction should not be used to pull the drops over.

After the dropping tip has been cleaned, V is fitted on to the brass stopper and W is fitted on to V . The supply bottle which rests on K is put into place. The support K is temporarily prevented from slipping through the tube attached to the roof of the box by the collar C . By fastening C at the proper distance along K the level of the liquid in S may be adjusted to stand a few millimeters above the level of the tip surface from which the drops fall. By the application of suction at the end of the vent tube, enough liquid is forced into the capillary tube to fill it completely up to the tip surface where the drops are formed. Due to capillary forces the liquid will remain in the capillary tube as long as desired. If the level of the liquid in S is not properly adjusted as described above, the liquid in the capillary will siphon back into S , or else drops will siphon over from S into W , neither of which is desired until the apparatus has been in the thermostat long enough to attain the proper temperature. The vent-tube weighing bottle is next adjusted on its stopper. After attaching the lower part of the box B by means of the wing nuts shown in the diagram, the apparatus is immersed in the thermostat. The apparatus is then leveled, the microscope stand is lowered into place, and the rod R is connected to K by means of C . In mov-

¹⁴ Any remaining correction due to vaporization loss is taken care of by determining the difference in weight between 30 drops and 5 drops of the liquid obtained under exactly similar conditions, according to the procedure of Morgan.

¹⁵ *J. Am. Chem. Soc.*, 41, 507 (1919); see also *ibid.*, 38, 248 (1916).

improbable that such a potential would have a sufficiently high value to explain the very great discrepancy.

A possible source of considerable error may be ascribed to the experimental method. The bubbles expand and contract as they pass along the tube. If this occurs after a tightly packed film has been formed, a contraction of the bubble may crumple the film and an expansion rupture it. This last effect would allow an additional adsorption. It does not seem very probable that the magnitude of this excess adsorption would be sufficient to explain the lack of agreement of the two methods.

The number of molecules of stearic acid in a monomolecular film on water is 5×10^{14} per sq. cm. as determined by the direct method. Our preliminary results give 8×10^{14} molecules of amyl alcohol in the film of this substance.

IV. THE RING METHOD FOR THE DETERMINATION OF SURFACE TENSION

(WITH H. F. JORDAN)

The ring method for the determination of surface tension is so convenient and so widely used that it is important to learn how to calculate the surface tension of a liquid from the total maximum pull (W) exerted between the liquid and the ring. Let P be defined by the equation

$$P = \frac{Wg}{4\pi R} \quad [1]$$

It is obvious that the surface tension (γ) is given by the identity

$$\gamma \equiv P \frac{\gamma}{P} \quad [2]$$

Some values of the factor γ/P were determined by Harkins, Young and Cheng, and were found to give curves of the type presented in Figure 4.

In the preliminary work reported here, many more determinations were made with numerous additional precautions. The new values are plotted in Figures 4 and 5.

Figure 4 represents a curved surface which gives the values of γ/P as a function of R^3/V and of R/r . Here R is the mean radius of the wire of the ring, and V is the value of Wg/ρ , in which ρ is the density of the liquid. Thus V is the volume of liquid lifted by the pull of the ring above the level of the plane portion of the sufficiently large surface of the liquid. In Figure 5 the coordinates have been changed in such a

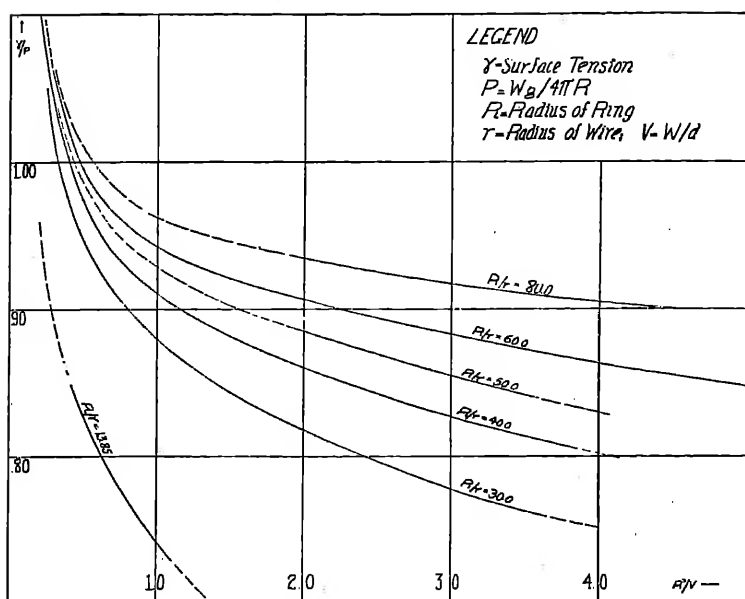


FIG. 4.—Correction Curves for the Ring Method for the Determination of Surface Tension.

Ordinates = γ/P .

Abscissæ = R^2/V .

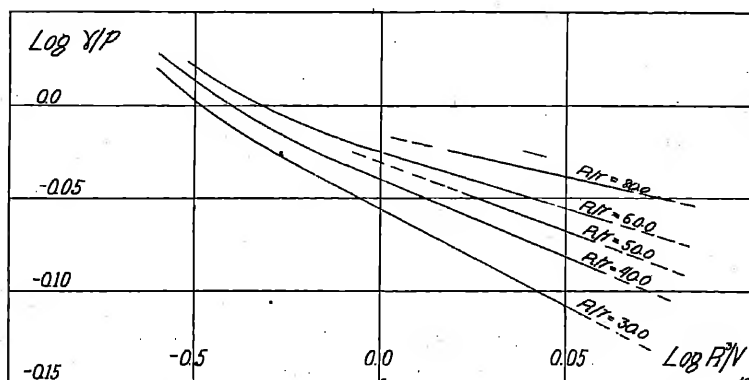


FIG. 5.—Correction Curves for the Ring Method for the Determination of Surface Tension.

way as to give a surface which is more nearly plane. The experimental method was improved in the following ways:

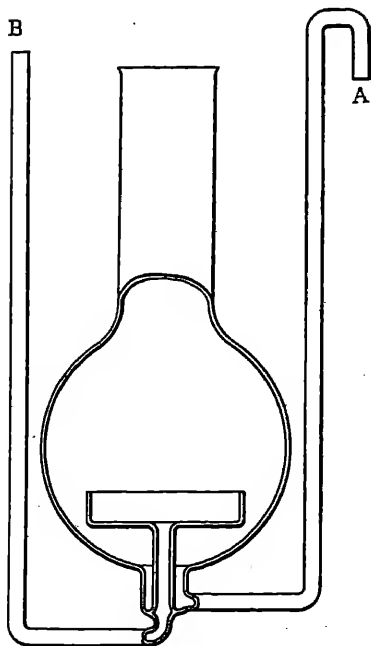


FIG. 6.—Shallow vessel for holding a liquid for the determination of its surface tension by the ring method. The outer flask allows this vessel to be held below the surface of the liquid in a thermostat. *A*, inlet for liquid; *B*, outlet for drawing off excess of liquid.

1. The liquid is held in a wide shallow glass vessel in a large spherical flask. This has a long neck. The flask, with the exception of the top of its neck, is immersed in the water of a thermostat. The shallow glass vessel is filled completely with the liquid whose surface tension is to be determined, and more of the liquid is added and allowed to overflow until a clear surface is formed. The liquid which overflows is drawn out by a tube from the bottom of the flask.

2. The pull was determined by a chainomatic balance sensitive to 0.05 mg.

3. Since lowering the surface of a liquid away from the ring sets up surface waves, the ring was pulled upward away from the surface by raising the balance. In order to do this the balance was mounted on a metal platform which was raised and lowered by a system of gears which worked with such ease and smoothness as not to impart momentum to the beam of the balance.

4. It was found necessary to support the wire of the larger plat-

inum-iridium rings of fine wire in at least four places, so a double stirrup was used.

5. It was found essential to adjust the ring carefully so that its plane is parallel to that of the surface of the liquid.

6. The vessel which contained the liquid had an area so large that no corrections need be applied for the curvature of the surface. The use of vessels of small area introduces a considerable error.

*University of Chicago,
Chicago, Illinois.*

SURFACE CONDUCTANCE ¹

By DAVID R. BRIGGS ²

Conductance of an electric current by the surface or interfacial phase of a liquid-solid system has largely been disregarded as unimportant or insignificant when compared with the conductance through the other phases of such a system. Isolated observations of surface conductance are recorded, although no systematic study of the phenomenon has been made. J. Stock ³ noted that quartz powder in such liquids as nitrobenzene and aniline greatly increased the apparent conductivities of these liquids. McBain ⁴ mentions experiments of Darke on the conductivity of dilute potassium chloride in a quartz capillary which showed a greater conductivity than the same solution in bulk. Fairbrother and Mastin ⁵ performed experiments with dilute solutions in a porous diaphragm which indicated that the specific conductivity of such solutions was increased in the diaphragm. Stamm ⁶ found that water used in electroendosmosis experiments on wood had a specific conductivity of 5.4×10^{-9} mho in bulk, while wood impregnated with the same water had a specific conductivity of 8.3×10^{-9} , from which he concluded that the water had become two to three times as conductive when in the wood as it was when in bulk. Briggs ⁷ in determining the ζ -potential on cellulose by the streaming potential method found that in diaphragms of this material surface conductance played a very important role and had to be measured before ζ could be calculated. He found in some instances that distilled water had 60 times as much capacity for conducting electricity in the diaphragm as it had in bulk.

Smoluchowski, ⁸ in 1905, pointed out that with very dilute solutions, surface conductance might become important in electrokinetic measurements and calculations. He defined as the mechanism by which

¹ Contribution from the laboratories of the Division of Agricultural Biochemistry, University of Minnesota. Published with the approval of the Director, as Paper No. 785, Journal Series, Minnesota Agricultural Experiment Station.

² Fellow, National Research Council.

³ Stock, *Ans. Akad. Wiss. Krakov. (A)*, p. 635 (1912).

⁴ McBain, (Discussion) Joint Symposium of Faraday and Physics Soc., p. 150 (1921).

⁵ Fairbrother and Mastin, *J. Chem. Soc.*, 125, 2319 (1924).

⁶ Stamm, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 246.

⁷ Briggs, *J. Phys. Chem.*, 32, 641 (1928).

⁸ Smoluchowski, *Physik. Z.*, 6, 529 (1905).

surface conductance is brought about, the motion of the ions making up the double layers toward the electrodes of opposite charge. The velocity, v , of this movement of the layer is inversely proportional to the viscosity coefficient of the liquid, η , and directly proportional to the charge, e , on the surface and to the potential gradient, $\partial V/\partial X$, along the direction in which the current would be conducted.

$$v = \frac{1}{\eta} e \frac{\partial V}{\partial X}$$

When the double layer is considered as a condenser (as is customary), e may be defined in terms of the potential across the two plates of the condenser, ζ , the dielectric constant, ϵ , and the distance between the plates, δ , as follows:

$$e = \frac{\zeta \epsilon}{4\pi \delta}$$

The current, I_s , carried across a cross-section of a diaphragm in the surface layer will equal the product of the velocity of motion, v , the density of charge on the surface, e , and the length of the surface, S , (corresponding to the circumference of a tube, if a tube instead of a diaphragm were being considered), *i.e.*,

$$I_s = veS = \frac{1}{\eta} e^2 S \frac{\partial V}{\partial X} = \frac{S}{\eta} \left(\frac{\zeta \epsilon}{4\pi \delta} \right)^2 \frac{\partial V}{\partial X}$$

The amount of current carried across the same cross section of the diaphragm by the liquid under the same potential gradient will equal the product of the area of cross section, A , of the liquid phase and the specific conductivity, κ , of the liquid, *i.e.*,

$$I_A = A\kappa \frac{\partial V}{\partial X}$$

The ratio of the current conducted by the surface to that conducted by the liquid will be, then,

$$\frac{I_s}{I_A} = \frac{S}{A\eta\kappa} \cdot \left(\frac{\zeta \epsilon}{4\pi \delta} \right)^2$$

From this equation it follows that if the specific conductivity of the interstitial liquid in a diaphragm is low or if the specific surface area of the diaphragm material is high, the ratio of I_s to I_A will be relatively

high and surface conductance will have to be taken into consideration in all electrokinetic calculations.

It is possible to measure the ratio I_B/I_A for any diaphragm permeated by a liquid in the following manner. A diaphragm of the material to be studied, *e.g.*, a pad of cellulose fibers, may be packed tightly between two porous gold plates in a cell similar to the one illustrated in Figure 1. These gold disks act as electrodes in a conductivity cell, while their

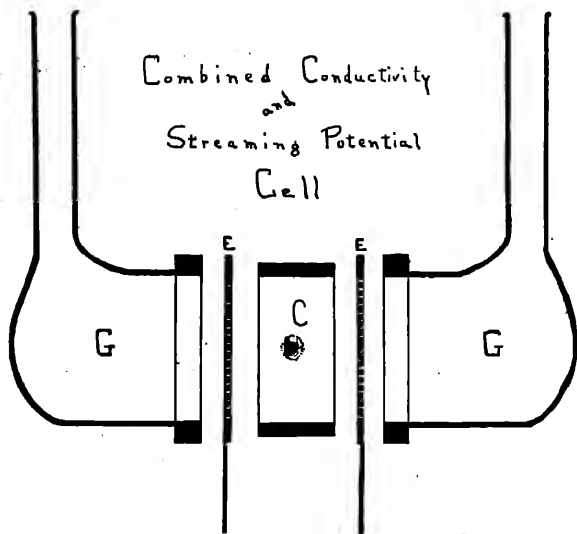


FIG. 1.

porosity makes it possible to replace the liquid inside the diaphragm without disturbing the diaphragm material itself, by streaming new liquid through the cell. The specific conductivity of the liquid as it exists inside of the diaphragm, κ_s , can be calculated from measurements of the resistance across the diaphragm while it is present, and from the subsequently determined cell constant of the diaphragm with a solution of concentration high enough so that surface conductance no longer plays a significant role. This value, κ_s , will be the sum of the conductivity due to the liquid and that due to the surface layer. The difference between κ_s and κ will be a measure of the surface conductance for a given diaphragm, then, and

$$\frac{\kappa_s - \kappa}{\kappa} = \frac{I_B}{I_A}$$

The equation of Smoluchowski states that when the ζ -potential becomes zero, the surface conductance must become zero also. From his equation one would expect that when the value for ζ varies, the value of I_s should vary in the same manner. That this is not true is amply illus-

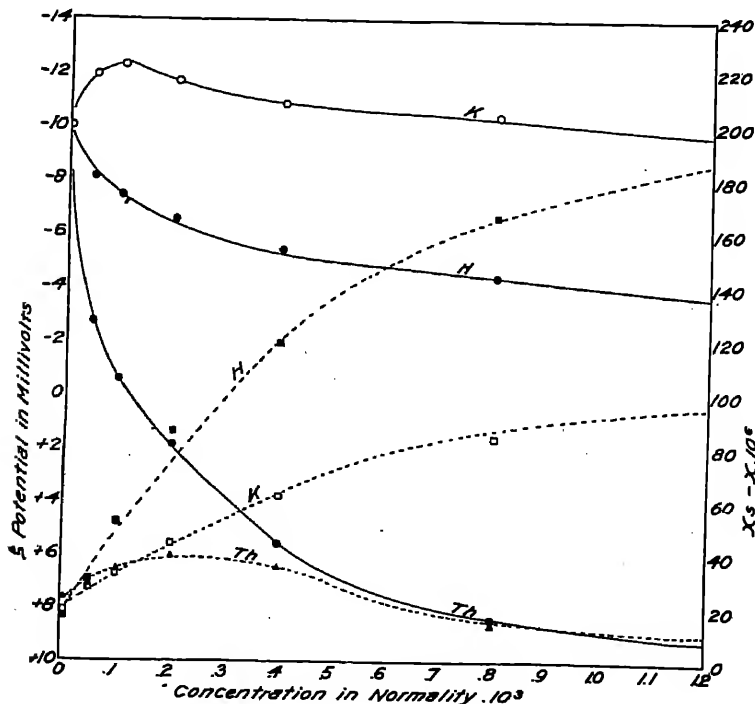


FIG. 2.—Showing that surface conductance ($\kappa_s - \kappa$) is not a function of the ζ -potential for cellulose membrane against salt solutions of varying concentration.

ζ -Potential curve—solid line.

Surface conductance curve—broken line.

trated by data shown graphically in Figure 2. Thorium chloride at very low concentrations lowers the negative charge on cellulose to zero potential and then as quickly builds up the charge of opposite potential to a high value. The corresponding values for the surface conductance for the same diaphragm and same solutions show no indication of becoming zero when ζ is zero but do become virtually insignificant while ζ has

still a high positive value. Again, for solutions of alkali metal cations the ζ -potential first increases, then decreases with increased concentration, but the actual amount of current conducted by the surface layer continues to increase in greater concentrations of the salts. Against solutions of H^+ ions, ζ steadily decreases, while the surface conductance increases with concentration. Thus, it appears that the surface conductance is not a function of the ζ -potential at all, and that the surface current is not due to movement of the surface layers of ions which give rise to the ζ -potential. Smoluchowski's equation then is incorrect, because his assumption that surface current is so carried is false.

To assume that the ζ -potential does not correspond to the adsorption force acting at the interface, might explain how the surface conductance could increase while ζ decreases (as with H^+ and alkali salts) with increased concentration of the salt solution. We could say then that the concentration of ions in the interface phase was increasing but that the difference in potential across the movable and immovable layers of liquid (*i.e.*, the ζ -potential) might vary independently of this surface concentration of salt ions. But such a picture would not explain why, in the case of thorium chloride solutions, the ζ -potential remains highly positive while the surface conductance diminishes to nearly zero. The fact that the value for ζ continues to increase indicates an increased concentration of Th^{+++} ions in the surface phase with increased concentration in the liquid phase. If surface conductance were an ionic conductance, its value should increase with increased concentration of ions in the surface-layer in all cases. The fact that this does not hold in the case of thorium salts can be taken to indicate that surface conductance is not ionic conductance, due to ions derived from the salts, at any rate, but has, rather, the nature of electronic conductance or of conductance due to the presence in the interface phase of a form of water which conducts the electric current.

At an interface, forces are acting upon the water molecules which serve to orient them and compress them into a smaller volume than they occupy in the bulk of the liquid. Under such conditions the equilibrium between the polyhydrols $[(H_2O)_2]$ and monohydrol may be shifted, so that monohydrol may make up, almost entirely, the surface phase. Kling and Lassieur⁹ have suggested that the monohydrol is a conductor of electricity, while the polyhydrols are not. In the interface phase the oriented layer of water molecules, which Harkins¹⁰ has said to be monomolecular, may be made up largely of monohydrol. The effects of

⁹ Kling and Lassieur, *Compt. rend.*, 177, 109 (1923).

¹⁰ Harkins and McLaughlin, *J. Am. Chem. Soc.*, 47, 2083 (1925).

salts upon the surface conductance would then be a function of their effects upon this water equilibrium. Bancroft¹¹ supposed the solubility of gelatin in water to be a function of the amount of hydrol (mono) present in the water. Increase in temperature increases this solubility by increasing the hydrol concentration in water. Polyvalent salts like those of aluminum and thorium in small amounts decrease the solubility of gelatin in water, presumably by decreasing the hydrol concentration in water. These same ions decrease the surface conductance to a small value at very low concentrations.

The current, I_s , which will flow through the surface phase of a cross section of a diaphragm, then, will be a function of the specific surface area of the diaphragm material, S , and will be governed by the specific nature of the solid phase and by the nature of the materials dissolved in the liquid phase, *i.e.*,

$$I_s = SC_sC_L$$

where C_s is a value defining the effect upon the water equilibrium in the interface phase (and therefore in the conducting capacity of that phase) for a given solid material, of which the diaphragm may be made, and C_L a value defining the same effect due to the liquid phase. C_s should remain constant for a given chemical individual, and C_L will be constant for a given solution.

$$\frac{I_s}{I_A} = \frac{\kappa_s - \kappa}{\kappa} = \frac{SC_sC_L}{A\kappa}$$

For a given diaphragm material, relative values for C_L with different salt solutions may be obtained when equal weights of the material are confined to the same volume and the various salt solutions are forced into the interstitial spaces. In such a case, S , C_s , and A would be constant and the value $\kappa_s - \kappa$ would be proportional to C_L . Table I and Figure 3 illustrate the relative values of $\kappa_s - \kappa$ for solutions of various salts at a cellulose-water interface in diaphragms containing approximately 0.40 gram of cellulose (S. and S. filter paper, No. 589) per cubic centimeter. As the table shows, the weights of the diaphragms per unit volume are not exactly the same but they are sufficiently identical, when corrected to bring the diaphragms to the same weights of cellulose per unit volume, to give comparable values of $\kappa_s - \kappa$. It will be noted that cations of equal valence show a definite lyotropic series which

¹¹ Bancroft, *J. Phys. Chem.*, 30, 1194 (1926).

TABLE I. *Effects of Various Cation Chlorides on the Surface Conductance in a Cellulose-Water Diaphragm.*

Salt Concentration	$\kappa \cdot 10^8$	$\kappa_s \cdot 10^8$	$(\kappa_s - \kappa) \cdot 10^8$	$(\kappa_s - \kappa) \cdot 10^8 \times \frac{0.40}{\text{g./cc.}}$	$A = \text{wt. of diaphragm}$ $B = \text{wt. of d.aphragm}$ per cc. $C = \text{cell constant}$
Normality	mho	mho	mho	mho	
KCl					
0.00000	2.3	20.3	18.0	18.2	$A = 6.914 \text{ grams}$ $B = .395 \text{ "}$ $C = .426$ $\frac{A}{C} = 16.25$
.00005	11.3	39.0	27.7	28.0	
.00010	17.4	49.7	32.3	32.7	
.00020	31.3	74.5	43.2	43.7	
.00040	61.0	122.0	61.0	61.7	
.00080	119.0	201.7	82.7	83.7	
.00160	236.5	346.0	109.5	110.8	
NaCl					
0.00000	1.6	21.3	19.7	19.8	$A = 6.946 \text{ grams}$ $B = .397 \text{ "}$ $C = .431$ $\frac{A}{C} = 16.15$
.00005	7.8	33.3	25.5	25.7	
.00010	14.4	43.2	28.8	29.0	
.00020	26.7	62.2	35.5	35.7	
.00040	51.6	97.5	45.9	46.2	
.00080	101.8	153.5	51.7	52.1	
.00160	199.0	270.0	71.0	71.4	
LiCl					
0.00000	1.9	22.2	20.3	20.5	$A = 6.920 \text{ grams}$ $B = .3965 \text{ "}$ $C = .440$ $\frac{A}{C} = 15.75$
.00005	7.3	30.6	23.3	23.5	
.00010	12.9	38.4	25.5	25.8	
.00020	23.8	54.4	30.6	30.9	
.00040	46.6	82.5	35.9	36.3	
.00080	91.9	138.0	46.1	46.6	
.00160	176.0	229.0	53.0	53.5	
CsCl					
0.00000	2.1	17.7	15.6	16.4	$A = 6.653 \text{ grams}$ $B = .380 \text{ "}$ $C = .415$ $\frac{A}{C} = 16.05$
.00005	9.7	35.8	26.1	27.4	
.00010	17.5	51.2	33.7	35.4	
.00020	30.9	72.0	41.1	43.2	
.00040	60.4	117.5	57.1	60.0	
.00080	119.0	192.0	73.0	76.7	
.00160	235.0	336.0	101.0	106.0	
HCl					
0.00000	2.4	18.2	15.8	16.7	$A = 6.635 \text{ grams}$ $B = .379 \text{ "}$ $C = .423$ $\frac{A}{C} = 15.70$
.00005	17.3	46.2	28.9	30.5	
.00010	38.5	88.2	49.7	52.4	
.00020	79.0	160.8	81.8	86.2	
.00040	158.0	271.0	113.0	119.0	
.00080	315.0	473.0	158.0	166.5	
.00200	802.0	1015.0	213.0	225.5	
.00400	1572.0	1800.0	228.0	240.5	
.01000	3880.0	4160.0	280.0	295.0	

TABLE I. (Continued.)

Salt Con- centration	$\kappa \cdot 10^8$	$\kappa_s \cdot 10^8$	$(\kappa_s - \kappa) \cdot 10^8$	$(\kappa_s - \kappa) \cdot 10^8 \times \frac{0.40}{\text{g./cc.}}$	$A = \text{wt. of diaphragm}$ $B = \text{wt. of diaphragm}$ $C = \text{cell constant}$ per cc.
Normality	<i>mho</i>	<i>mho</i>	<i>mho</i>	<i>mho</i>	
NH₄Cl					
0.00000	2.8	23.4	20.6	20.6	$A = 6.978 \text{ grams}$ $B = .3985 \text{ "}$ $C = .415$ $\frac{A}{C} = 16.80$
.00005	9.6	41.3	31.7	31.8	
.00010	16.4	56.1	39.7	39.8	
.00020	31.0	80.3	49.3	49.4	
.00040	59.2	124.0	64.8	64.9	
.00080	116.0	204.5	88.5	88.7	
.00160	229.0	345.0	116.0	116.4	
BaCl₂					
0.00000	1.8	21.3	19.5	19.1	$A = 7.162 \text{ grams}$ $B = .4095 \text{ "}$ $C = .449$ $\frac{A}{C} = 15.95$
.00005	8.2	39.7	31.5	30.8	
.00010	15.4	49.6	34.2	33.4	
.00020	29.0	65.8	36.8	36.0	
.00040	56.1	98.5	42.4	41.4	
.00080	108.7	150.0	41.3	40.3	
.00160	213.0	265.0	52.0	50.8	
MgCl₂					
0.00000	2.3	24.0	21.7	21.3	$A = 7.130 \text{ grams}$ $B = .407 \text{ "}$ $C = .435$ $\frac{A}{C} = 16.40$
.00005	8.4	32.6	24.2	23.7	
.00010	14.2	39.8	25.6	25.1	
.00020	26.9	57.8	30.9	30.4	
.00040	51.5	87.2	35.7	35.1	
.00080	98.7	136.0	37.3	36.6	
.00160	192.0	232.0	40.0	39.3	
AlCl₃					
0.00000	1.8	29.8	28.0	23.6	$A = 8.295 \text{ grams}$ $B = .474 \text{ "}$ $C = .541$ $\frac{A}{C} = 15.35$
.00005	9.9	46.9	37.0	31.2	
.00010	16.6	58.2	41.6	35.1	
.00020	31.2	78.9	47.7	40.2	
.00040	57.9	96.4	38.5	32.5	
.00080	109.6	139.0	29.4	24.8	
.00160	209.0	224.0	15.0	12.6	
.00320	414.0	423.0	9.0	7.6	
ThCl₄					
0.00000	2.4	27.5	25.1	23.5	$A = 7.478 \text{ grams}$ $B = .428 \text{ "}$ $C = .460$ $\frac{A}{C} = 16.20$
.00005	9.8	39.9	30.1	28.2	
.00010	20.1	54.6	34.5	34.0	
.00020	37.5	80.0	42.5	39.8	
.00040	68.4	106.2	37.8	35.6	
.00080	115.0	130.0	15.0	14.0	
.00160	199.0	212.0	13.0	12.2	
.00320	323.0	336.0	13.0	12.2	

follows very closely the order of mobilities of these ions. (Ammonium and caesium ions are reversed in their relation to potassium from a mobility standpoint. If the whole series were repeated and exactly the same weights of diaphragm material used in every case to fill the standard cell compartment, these ions would probably take their proper order in the lyotropic series. In the present case, the data were taken from an experiment not devised primarily to study surface conductance, and the values for ammonium, caesium, and potassium ions may be taken to be

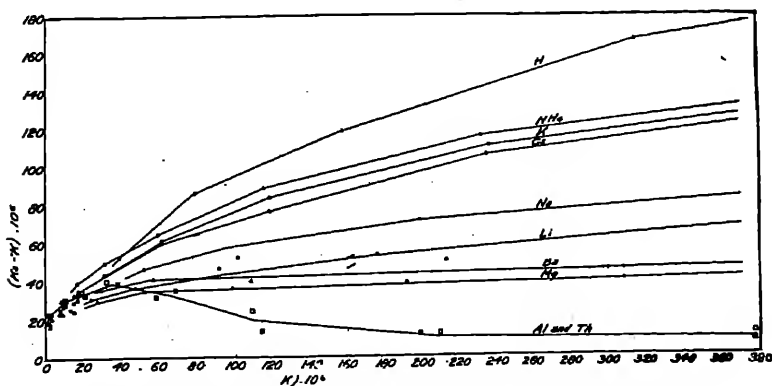


FIG. 3.—Illustrating the valence and lyotropic effect of cations upon the surface conductance of a cellulose membrane.

the same within experimental error.) It may be pointed out that with salts of monovalent cations the surface conductance continues to increase in amount as the concentration increases, and only at high concentrations (of the order of 0.1 N to 5 N) will the surface conductance become insignificant as compared to the conductance due to the liquid inside the diaphragm pores. Of course, the concentration at which this condition will be attained depends upon the amount of diaphragm material packed into unit space and upon the specific surface area of this material. With salts of bivalent cations, surface conductance becomes insignificant at a much lower concentration of salt, while with tri- and tetra-valent salts it becomes insignificant (though still recognizable) at very low concentrations of salt (above 0.0004 N it is insignificant as compared to the bulk conductance).

That surface conductance seemed to be a function of the colloidity of a substance and therefore proportional to the specific surface area of

that substance was indicated by results obtained from ζ -potential determinations on the assortment of powdered or fibrous materials against distilled water, as shown in Table II.

TABLE II. ζ -Potential and Relative Surface Conductance of Various Diaphragms Against Distilled Water.

Diaphragm Material	$(\kappa_s - \kappa) \cdot 10^6$				ζ	Cell Constant of Diaphragm
	$\kappa \cdot 10^6$	$\kappa_s \cdot 10^6$	$(\kappa_s - \kappa) \cdot 10^6$	cell constant		
	<i>mho</i>	<i>mho</i>	<i>mho</i>	<i>mho</i>	<i>mv.</i>	
Alumina (Al_2O_3) ...	2.6	3.0	0.4	0.8	+19.3	0.515
Talc	2.3	7.0	4.7	6.7	-19.5	0.697
Quartz powder	2.7	6.4	3.7	5.5	-19.3	0.675
Silica gel (Patrick's)	2.1	51.6	49.5	82.5	-22.2	0.600
Silica gel (116 per cent porosity)	3.2	61.2	58.0	156.0	- 3.2	0.375
Ground glass	2.7	13.2	10.5	15.1	-32.3	0.693
Sulfur (Flowers) ...	2.6	5.3	2.7	2.5	-81.1	1.059
Starch	2.3	19.6	17.3	25.2	-57.9	0.686
Asbestos	2.7	10.1	7.4	24.4	-21.6	0.303

Since the diaphragms shown in Table II were confined in a cell of constant dimensions (volume = 17.6 cc.), the cell constants may be taken as inverse measures of the pore space present in the diaphragms. It is desirable to obtain values of $(\kappa_s - \kappa)$ in the diaphragms when the pore spaces (and, therefore, the volume occupied by the solid) are constant. This is calculated in Table II, Column 5, by multiplying $(\kappa_s - \kappa)$ by the reciprocal of the cell constant of the diaphragm.

In Table III are given the surface conductance measurements on a series of silicon dioxide diaphragms. The quartz diaphragm was of crystalline quartz ground to pass through a 200 mesh screen. The first silica gel is one prepared by the method of Patrick¹² and is less porous than the last two prepared by Holmes¹³ and having 116 per cent and 140 per cent porosity values determined by his method. To obtain these surface conductances $(\kappa_s - \kappa)$ on a comparable basis, the observed value is multiplied by the reciprocal of the cell constant of the diaphragm as found with 0.15 N KCl solution. Data shown in Table III indicate that surface conductance is roughly proportional to the surface area of the material, but the results are not of sufficient scope to make it possible to draw from them a more definite conclusion relative to the exact functional relation that surface conductance bears to the specific surface area of a material.

¹² Patrick, U. S. Pat. 1,297,724 (March 18, 1919) and 1,335,348 (March 30, 1920).

¹³ Holmes, *Ind. Eng. Chem.*, 17, 280 (1925).

TABLE III. *Surface Conductance for Diaphragms of Silicon Dioxide of Varying States of Colloidal Aggregation.*

Salt Conc.	$\kappa \cdot 10^6$	$\kappa_s \cdot 10^6$	$(\kappa_s - \kappa) \cdot 10^6$	$\frac{(\kappa_s - \kappa) \cdot 10^6}{\text{cell constant}}$	Cell Constant	Dry Wt. of Diaphragm Grams
Normality	<i>mho</i>	<i>mho</i>	<i>mho</i>	<i>mho</i>		
Quartz Powder.						
0.00005	13.7	22.1	8.4	11.8	0.716	27.144
.00010	19.2	30.1	10.9	15.2		
.00020	34.5	48.5	14.0	19.5		
.00040	65.0	82.5	17.5	24.4		
.00100	155.0	188.0	23.0	32.1		
.00200	335.0	360.0	25.0	34.9		
Silica Gel (Patrick's).						
0.00005	12.1	93.8	81.7	143.4	0.570	14.245
.00010	19.2	117.0	97.8	171.7		
.00020	33.6	156.0	122.4	214.8		
.00040	65.8	223.0	157.2	276.0		
.00100	153.0	363.0	210.0	369.0		
.00200	302.0	570.0	268.0	470.5		
Silica Gel (Holmes, 116%).						
0.00005	12.2	96.0	83.8	223.5	0.375	5.988
.00040	65.2	196.0	130.8	349.0		
.00200	313.0	542.0	229.0	611.0		
Silica Gel (Holmes, 140%).						
0.00010	20.8	99.5	78.7	303.0	0.260	7.030
.00100	159.0	243.0	84.0	323.0		
.00200	315.0	411.0	96.0	369.0		

SUMMARY

In aqueous solutions of low specific conductivity present in the interstices of a diaphragm material, *e.g.*, a pad of pure paper pulp, the electrical conductance through the interface phase is much greater than that through an equal volume of the liquid in bulk. This fact has been largely neglected in calculations of the ζ -potential at interfaces when electrokinetic technic was used.

A method is offered by which the surface conductance of diaphragms may be determined.

Surface conductance is important in electrokinetic determinations only when the solutions used have low specific conductivities or when the micellar material has a high specific surface area.

Salt solutions show definite valence and lyotropic effects upon the surface conductance.

Surface conductance is not a function of the ζ -potential.

Surface conductance measurements possibly may be utilized to obtain values of the relative specific surface areas (*i.e.*, colloidalilty) of materials.

*University of Minnesota,
St. Paul, Minn.*

THE EFFECT OF ADSORBED WATER ON THE ELECTRICAL CONDUCTIVITY OF POWDERS

BY FRANK B. KENRICK AND F. J. GIFFEN

Previous work in this laboratory has shown that certain solids such as boric acid have a marked ability to initiate bubbles in solutions highly supersaturated with respect to oxygen, while other materials, including preheated glass, in tubes of which the experiments were carried out, do not possess this property.¹ That the bubbles were not initiated by gas films on the solids was definitely proved in many cases by the fact that the solids were produced, where this was possible, from solutions which were supersaturated with respect to these solids and previously saturated with oxygen at a pressure of 30 atmospheres, but kept at 70 atmospheres during the formation of the solid. The solutions were thus unsaturated with oxygen while the solids were coming into existence. When the solids could not be produced in this way and had to be introduced from the outside into the oxygen solutions it was found that the majority of these, although they at first initiated bubbles, lost this property after they had been for 24 hours in contact with the oxygen solution saturated at 30 atmospheres and kept at 70 atmospheres. It was considered probable, therefore, that those solids which did not lose the ability to initiate bubbles under this treatment were also free from films of air. Such results however are obviously less reliable than those obtained with materials which could be grown in the solution. Although comparatively few cases have as yet been studied, the general character of the materials suggested that the property of initiating bubbles might be related to the ease with which the solid and liquid surfaces can be separated, and consequently to non-wettability or to low adsorption of water vapor.

Some unpublished experiments of Farncombe showed that with an apparatus similar to McBain's sorption balance² boric acid did not adsorb appreciable amounts of water vapor even when the vapor pressure approached the saturation value, while glass powder gave results of adsorption comparable with those found by other investigators. Although this apparatus was not very delicate the result encouraged us to examine a wider range of materials. Direct determinations of adsorption are rather difficult to carry out, and it was in the hope of finding an easier

¹ Farncombe, *Trans. Roy. Soc. Can.*, 19, 32 (1925).

² McBain and Baker, *J. Am. Chem. Soc.*, 48, 690 (1926).

way of picking out materials which might be expected to start bubbles that we made the conductivity experiments described in this preliminary report.³

Our method consisted in measuring, by means of an electroscope, the rate of discharge of a condenser through the powder under various conditions of moisture. Most of the experiments were made with a small Leyden jar of about 0.002 microfarads capacity. This was connected to a tin-foil electroscope and charged to a sparking distance of about 3 mm. with an induction coil. The powdered solids were packed to a depth of about 1 cm. in a fused silica tube of 1 cm. diameter between two plugs made by rolling up a strip of copper gauze to which electrical connection was made by wires. The ends of the tube were fitted with corks carrying the wires, and glass tubes by means of which either dry or moist air could be passed through the powder. The rate of leakage of the charge through the powder was determined under various conditions by timing the fall of the electroscope leaves over a fixed range on the scale.

The air was dried by passage over calcium chloride and phosphorus pentoxide. Air containing water vapor of known concentration was obtained by bubbling ordinary air through Geissler tubes of water in a thermostat and then passing it through a U-tube packed with cotton wool in the same thermostat. The powders were first dried by passing dry air through the tube for about half an hour or until there was no appreciable leakage of current through the tube. (Lead chloride, manganese dioxide and silver iodide conducted well even after 24 hours' drying, and therefore could not be investigated by this method.) Air of various degrees of moisture was then passed through the powder for various lengths of time, until a maximum conductivity was reached. From half an hour to an hour was generally sufficient.

It was found that some powders, glass for example, conducted with even small concentrations of water vapor and that the conductivity increased as the partial pressure of the water vapor approached the saturation value, while others, such as salicylic acid, remained practically non-conducting until the saturation point was nearly reached, when, as might be expected, all powders conducted well.

It is realized that many points will have to be studied more carefully before definite conclusions can be drawn. The fineness of the powder, and the method of packing and making the end-connections have an obvious influence; also the leakage along the walls of the tube and the relative conductivity of the powders which we have classed as non-conducting by this rough way of measuring can be determined

³H. L. Curtis has determined surface resistance of a number of solid dielectrics in block form, *Bull. Bur. Standards* 11, 359 (1915).

with an improved apparatus. These points are at present under investigation.

The following table gives the qualitative results obtained with the apparatus described above, when dried powders were subjected to air bubbled through water at from 6° to 8° C., or about 12° C. below the temperature of the powder (room temperature) which varied from 18° C. to 20° C. The powders from *glass* to *talc* are arranged in order of decreasing conductivity; the remainder, *salicylic acid* to *sealing wax*, were practically non-conducting and are in arbitrary order. Opposite each is given the behavior of the substance in contact with aqueous solutions of oxygen (saturated at from 30 to 40 atmospheres pressure) when the pressure was reduced to one atmosphere.

In this table "*introduced*" means that the solid could not be produced in the solution, but was introduced into the tube and kept for at least 24 hours at 70 atmospheres. "*Produced*" means that the solid was produced in the unsaturated oxygen solution from a solution made supersaturated in respect to the solid either by cooling or by chemical precipitation.

TABLE I. *Comparison of Conductivity with Ability of Solids to Initiate Bubbles.*

Solids in Descending Order of Conductivity	Behavior in Contact with Supersaturated Oxygen Solutions
Powdered glass	<i>Introduced</i> ; no bubbles ^a
Barium chromate	Both <i>introduced</i> and <i>produced</i> ; no bubbles ^a
Chromic chloride (CrCl ₃)	<i>Introduced</i> ; violent bubbling at first, but less and less the longer at 70 atmospheres, but never no bubbles ^a
Quartz sand	<i>Introduced</i> ; no bubbles ^a
Precipitated calcium sulphate....	<i>Produced</i> ; bubbles ^a
Talc	<i>Introduced</i> ; free bubbling; not reduced by keep- ing longer at 70 atmospheres ^a
Salicylic acid	<i>Produced</i> ; free bubbling ^a
Flowers of sulfur.....	<i>Introduced</i> ; bubbling, but not very freely ^a (pre- cipitated sulfur, <i>produced</i> ; no bubbles) ^a
Boric acid	<i>Produced</i> ; violent bubbling, even when pressure was reduced by only 20 atmospheres ^a
Naphthalene	<i>Introduced</i> ; bubbles but not many ^a
Caffein	<i>Produced</i> ; bubbles ^a
Sealing wax	<i>Introduced</i> ; bubbles ^a

Although the data are not sufficiently reliable to warrant a final conclusion, it is seen that with the exception of chromic chloride (purple flakes) the materials which initiate bubbles are in the lower part of the list. The chromic chloride is of a very flaky structure, and having been

^a Farncombe, *Trans. Roy. Soc. Can.*, 19, 32 (1925); also some unpublished results.

^b Giffen.

"introduced" it is doubtful if all air films were got rid of in the bubbling experiments. The results with talc and naphthalene may be doubtful for the same reason. The conductivity result for calcium sulfate may be questioned because the dry air might have dehydrated enough of the salt to combine with the moisture subsequently introduced. Precipitated sulfur, which is probably non-crystalline can hardly be compared with the flowers of sulfur. It may be added that many other substances, both crystalline and otherwise, produce no bubbles, but these have not yet been investigated for surface conductivity.

It was noticed in the experiments with dried powdered glass that a few minutes after the current of moist air had been shut off, although the tube was closed, the conductivity fell off rapidly and then remained practically constant at a low value. A fresh supply of moist air restored the original conductivity, and on shutting off the air the conductivity again fell, but not so much as the first time. This could be repeated many times until finally the fall, when the air current was stopped, became comparatively slight. Conversely, if the glass were first subjected to moist air for 24 hours, and then to dry air for a few minutes, the conductivity fell to zero, but on shutting off the air the conductivity began to increase. This also could be repeated many times. This behavior suggests an equilibrium between a surface of adsorbed water and water dissolved in the glass.

This work is being continued with an improved electrical arrangement with a small sulfur condenser, low voltage (150 volts) and a very delicate aluminium leaf electroscope. The powders are packed in a glass U-tube lined with baked shellac, and the contacts are made with mercury instead of copper gauze.

*Chemical Department,
University of Toronto,
Toronto, Ontario, Canada.*

THE ACTIVITY AND ADSORPTION OF *p*-TOLUIDINE IN THE SURFACE OF ITS AQUEOUS SOLUTION

BY J. W. MCBAIN, W. F. K. WYNNE-JONES AND F. H. POLLARD

In a previous communication¹ it was shown that even unsaturated solutions of substances such as toluidine, amyl alcohol and camphor are covered with so much adsorbed material that it cannot find room in a monomolecular film but probably extends in chains of oriented molecules well down into the solution. Furthermore, this amount of adsorption is very distinctly greater than that predicted by the Gibbs theorem as ordinarily quoted, where adsorption is deduced from the change in surface tension with concentration.

There are three alternatives; either the discrepancy might be due to the use of concentration in place of the thermodynamic potential or activity as actually used by Gibbs and G. N. Lewis, or an extra term should be added in the Gibbs theorem to take account of the electrification which certainly occurs at all such surfaces as is evident from electrokinetic experiments, or, lastly, there may be some inherent error in the attempt to measure the equilibrium at the surface by means of any method which is not completely static. The experiments adduced differed from all previous ones, including those of Harkins and D. M. Gans,² in that the surfaces (bubbles) were bodily removed from the vessel containing the original solution before they were allowed to collapse, thus escaping the inevitable pumping action caused by the bubbling which unavoidably causes mixing of the various portions of the solution no matter how carefully constrictions, baffles, or orifices may be interposed. Such mixing is important when tens of thousands of bubbles are successively introduced into and withdrawn from an incompressible liquid filling a compartment; it causes all results to be too small unless the bubbles are removed to a separate vessel before being allowed to collapse. Hence all the previous results in the literature must be discarded as necessarily too low.

A fourth possible direction in which one might have searched for a source of the discrepancy was in the measurement of surface tension. However, this was very carefully measured by the Ferguson method.

¹ McBain and Davies, *J. Am. Chem. Soc.*, 49, 2230-54 (1927).

² Colloid Symposium Monograph, Vol. 5, Chemical Catalog Co., Inc., New York, 1928, pp. 40-42.

Further, the results can hardly be explained in this way since it is over nearly the whole range of concentrations that the observed sorption so greatly exceeds that predicted. It is easy in a single point of the surface tension curve to make an inaccurate determination of the slope, but it is not possible to do this along the whole curve without complete displacement of the latter. Thus in order to double the slope it would be necessary to double the lowering of surface tension for every concentration. This is not a practical possibility when the lowering of surface tension is already so great. Any redetermination of surface tension must result in only a minor adjustment. Through the kindness of Dr. Harkins we have been provided with a drop-weight tip for making such determinations.

The present note is confined to a discussion of the first alternative explanation and to a determination of the activity of aqueous *p*-toluidine. It is shown that for the particular case of *p*-toluidine the genuine Gibbs theorem gives exactly the same results as the concentration formula, and that this explanation cannot therefore explain the discrepancy. There remains a conflict between the recorded observations and the theorem of Gibbs in which he did not take electrification into account.

We have determined the partial pressure of *p*-toluidine over its aqueous solutions. This has enabled us to make use of the Gibbs equation No. 585 with its direct relation between partial pressures and adsorption.

EXPERIMENTAL

The method adopted is quiet distillation in an evacuated system. A small sample of distillate is allowed to collect from a very large amount of toluidine solution at 25° without ebullition (which causes spray) but with stirring. Analysis of this distillate gives the relative partial pressures of *p*-toluidine and water, and since that of water is accurately known the value for *p*-toluidine follows.

Dunn and Rideal³ have utilised the method of distillation in studying aqueous hydrochloric acid. Various criticisms of their procedure⁴ had to be avoided here. Condensation in the space between the distilling flask and the receiver must be wholly avoided. This is readily achieved by keeping all these exposed parts electrically heated. Distillation has to be continuous but not too rapid, so that thorough stirring may be effected between the evaporating surface and the bulk of the solution.

The apparatus is shown in Figure 1. A two-liter Jena flask, sealed at the top, is connected by means of a side tube 10 cm. long through a ground joint to a receiver made of Pyrex glass. The receiver is of about 30 cc. capacity and is closed at the bottom with a large Pyrex tap. The receiver was fitted with an ice jacket as shown, and also with a stopper

³ *J. Chem. Soc.*, 125, 676 (1924).

⁴ Dobson and Masson, *J. Chem. Soc.*, 125, 668 (1924); Hudleston, *ibid.*, p. 1558.

for use in weighing. The side tube leading from the distilling flask to the receiver was wide and as short as possible in order to lessen any diffusion effects. The distilling flask was fitted with heating coils consisting of nichrome wire wound on asbestos, and these were regulated by means of rheostats to about 25° – 26° C. in order to prevent condensa-

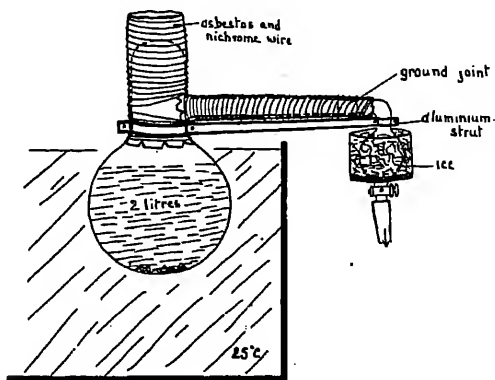


FIG. 1.

tion between them. The distilling flask and receiver were "strapped" together by means of aluminium straps and then clamped in a specially designed shaker, or rather, rocking machine. The shaker was immersed in a thermostat so that when the apparatus was being rocked the distilling flask was well covered; the neck and delivery tube which were out of the water were maintained at 25° by electrically heated coils. The thermostat was kept at a temperature 24.9° .

The *p*-toluidine was prepared from Kahlbaum's purest, by sublimation after mixing it with potassium hydroxide, and consisted of perfectly white crystals melting at 45.6° .⁵ In setting up an experiment the toluidine was dissolved in about two liters of boiled-out conductivity water and introduced into the distilling flask through the side tube whereupon the receiver was attached using the minimum amount of vacuum grease. The flask was allowed to remain overnight in the thermostat. Thereupon it was connected by means of pressure tubing to a trap immersed in a freezing mixture contained in a Dewar flask, thence to a monometer and a large reservoir and through a calcium chloride tube to a vacuum pump. Glycerol was used as a lubricant between the glass and rubber. Upon first evacuating violent ebullition took place but gradually ceased. Evacuation was continued for three or four hours. No condensation took place within the apparatus because the receiver jacket was filled with warm water. It is necessary to remove all air from the system if distillation is not to be too slow. When this was accomplished the tap

⁵ Compare Berliner and May, *J. Am. Chem. Soc.*, 49, 1007 (1927).

at the bottom of the receiver was closed and disconnected from the pumping system.

The shaker was started and the apparatus allowed to shake for half an hour in order that the vapor and solution might have a good opportunity to get into equilibrium. The shaker was then stopped, ice placed quickly in the jacket and the shaker was started again. It took from one to one and a half hours for about 5 cc. to distil, but distillation was stopped at various convenient times, so that the same amount of distillate was not collected each time. A small correction in the concentration of the distilling liquid would be required if more than about 10 cc. was collected, but usually not more than 5 cc. was collected. The apparatus was then taken out of the thermostat and the receiver removed. The ice jacket was taken off and the receiver (closed with a glass stopper and containing the distillate) was weighed. The distillate was then diluted so as to dissolve all *p*-toluidine and analysed by means of the Zeiss interferometer. A sample of the liquid remaining in the distilling flask was analysed in a similar manner. The interferometer was that used by G. P. Davis but it had been readjusted. Hence one division corresponded to 1.51×10^{-6} grams per 1 cc. solution instead of 1.49×10^{-6} grams per 1 gram solution as found by Davies.

The calculation of the results is illustrated by experiment 2 taken from the fifth line of Table 1. Here 12.6971 grams of solution from the distilling flask were diluted to 123.30 grams, and 4 readings were taken against water with two portions, giving 238.8, 238.7, 239.2, 239.0, mean 238.9 divisions. Hence the concentration of the toluidine solution was $123.30/12.697 \times 238.9 \times 1.51 \times 10^{-6} = 3.503$ grams *p*-toluidine per 1000 grams solution. The weight of distillate obtained from two liters of the above solution was 1.9047 which was diluted to 40.64 giving a reading 371.8 divisions whence its concentration was 12.00 grams of *p*-toluidine in 1000 grams of condensed vapors or 12.00 grams *p*-toluidine to 9.88 grams water. Since the vapor pressure of water at 24.9° is known to be 23.62 mm. and the lowering of vapor pressure in the very dilute solution of *p*-toluidine is negligible, the partial pressure of the *p*-toluidine is to that of water vapor in the proportion of the number of molecules of *p*-toluidine to molecules of water vapor. Hence the partial vapor pressure of the *p*-toluidine above a solution containing 3.503 grams *p*-toluidine in 1000 grams solution is

$$23.62 \times \frac{12.00}{988.0} \times \frac{18.016}{107.08} = 0.0482 \text{ mm.}$$

The results are collected in Table 1 where the first column gives the concentration of the solution and the last the ratio between the vapor pressure of the *p*-toluidine, as recorded in column 5, to the molar fraction of the original solution, as recorded in column 2. As is evident from

the value in column 2, owing to the comparative insolubility of *p*-toluidine most of the solutions are too dilute to lower the partial pressure of the water appreciably, although this is allowed for in the last two lines of the table, where it affects the partial pressure by only one unit in the third significant figure.

TABLE 1. *The Partial Vapor Pressure of p-Toluidine Above Its Aqueous Solution at 24.9°, that of Water Being 23.62.*

Grams per 1000 Grams Solution	Molar Fraction	Distillate Weight	Concn.*	Pressure mm. Hg	Ratio Col. 5: Col. 2
1.609	0.0002708	2.7156	5.646	0.0226	83.46
2.017	0.0003397	3.4249	6.885	0.0275	80.95
2.644	0.0004454	10.17	8.371†	0.0336	(75.44)
2.754	0.0004639	4.6719	9.322	0.0374	80.62
3.503	0.0005911	1.9047	12.00	0.0482	81.54
4.45	0.0007513	3.5867	15.03	0.0592	78.80
5.326	0.0008995	9.7819	19.01	0.07525	83.66
6.210	0.001050	1.5563	20.04	0.08124	77.37
				mean	80.9

* Grams *p*-toluidine per 1000 grams total distillate.

† There was some doubt about the concentration of this distillate.

DISCUSSION

The result of the measurements is to show that the partial pressure of *p*-toluidine above its aqueous solution is exactly proportional to the concentration, as is shown by the constancy of the ratio in the last column of Table 1. Hence the activity coefficient of the toluidine is a constant independent of the concentration.

Owing to this circumstance and also to the fact that all the partial pressures are so small that both the *p*-toluidine and the water vapors may be treated as perfect gases, it is for our purpose a matter of complete indifference what numerical value is assigned to the activity, or in other words, what standard state is chosen. Such a standard state could be taken in infinitely dilute solution or in the vapor above pure *p*-toluidine but the calculation of adsorption by the Gibbs theorem is completely unaffected by any such choices.

Gibbs himself developed the little-known equation No. 585 in which the adsorption is expressed directly in terms of partial pressure of the solute and the density of its vapor corresponding to that partial pressure, all purely thermodynamic properties being eliminated. This enables us to predict the adsorption directly from Table 1 using Gibbs' rigorous equation No. 585 without troubling to evaluate thermodynamic potential or activities. This equation reads:

$$\Gamma = -D \cdot \frac{d\sigma}{dp}$$

where σ is the surface tension, p the partial pressure of the toluidine and D the density of its vapor above the solution. The result is identical

with the original rigorous Gibbs theorem $\Gamma = -d\sigma/d\mu$ as well as with the usual inaccurate form $\Gamma = -c/RT \cdot d\sigma/dc$ or the Lewis and Randall expression $\Gamma = -a/RT \cdot d\sigma/da$ where c is the concentration of *p*-toluidine in the bulk of the solution and a and μ are its activity and potential, respectively.

Since the partial pressure is proportional to the concentration, Γ is independent of any agreement or lack of agreement with the requirements for an ideal solution. This is due to the fact that the density \bar{D} in the numerator increases in the same proportion as the dp in the denominator. Thus abnormally high increase of partial pressure as the *p*-toluidine is added to water is exactly offset in the formula by the corresponding increase in the amount and therefore density of the *p*-toluidine in the vapor.

The final result is then that the true formulas of Gibbs and Lewis as well as the concentration formula all give the same results; namely, that which was recorded in the calculations of McBain and Davies and which however is only half of that experimentally observed. They found that the amounts of *p*-toluidine, amyl alcohol, and camphor actually adsorbed are several times greater than the amount which can be close-packed into a monomolecular film. Likewise, McBain, Laing, Hillman and Harriott (now being published) have found that soap is adsorbed to at least twice the extent that can be accommodated in a monomolecular film. Most of the early data of W. C. M. Lewis show adsorption grossly exceeding that calculated from the inaccurate Gibbs concentration formula.

SUMMARY

Since it is now found that the partial pressure and activity of aqueous *p*-toluidine are proportional to the concentration, the two-fold conclusion of McBain and Davies is strengthened:—firstly, that the direct experimental observations show that in all these solutions of soluble substances, even when far from saturation, not only is the surface completely covered with a monomolecular film of the solute but that there is also a large excess in the neighborhood of the surface probably extending in the form of oriented molecules well into the solution. Secondly, that the thermodynamic treatment of Willard Gibbs which has been followed by all subsequent writers is insufficient, because it omits consideration of adsorption resulting from the admitted electrification of all such surfaces, as exhibited for instance in experiments on electrokinetics.

*Department of Chemistry,
Stanford University,
California.*

ADSORPTION OF SODIUM OLEATE AT THE AIR-WATER INTERFACE

By M. E. LAING, J. W. MCBAIN AND E. W. HARRISON

In a previous communication one of us¹ showed that the material comprising soap film contains more oleic acid than corresponds to neutral soap. Only in the presence of excess alkali does the adsorbed substance consist of neutral soap. The experiments were carried out on masses of bubbles of unknown area and there have been no data referring to known surfaces because the few data supplied by physicists were invalidated by the presence of carbonic acid which decomposes soap solutions.

The present paper supplies the first quantitative information obtained with exceptionally pure sodium oleate. The results are obtained by the method of McBain and Davies,² which consists in removing the soap bubbles from solution before allowing them to collapse and then carrying out a complete analysis of the foam after it has collapsed to liquid. The results confirm the findings of the previous paper and they show that more oleate is adsorbed than can possibly be accommodated in a single monomolecular layer.

The adsorption of soap is of exceptional interest for many reasons. For example according to the crude form of the Gibbs theorem relating concentration and surface tension it should not be adsorbed at all. Throughout the whole range of ordinary concentrations the surface tension is nearly constant but tends to rise with increase in concentration. Hence the slope is zero or negative and the adsorption should be likewise zero or negative instead of actually being exceptionally prominent. This contradiction is all the more remarkable because soap has lowered the surface tension of water by no less than 60 per cent.

It is our intention in this series of studies to elucidate this problem and to present evidence leading to a definite picture of the structure of such surfaces and the neighboring layers of solution.

PREVIOUS WORK

All experiments here recorded were carried out in the Bristol Laboratory. In preliminary work by T. R. Bolam purified nitrogen

¹ Laing, *Proc. Roy. Soc.*, 109, 28-34 (1925).

² *J. Am. Chem. Soc.*, 49, 2230-2254 (1927).

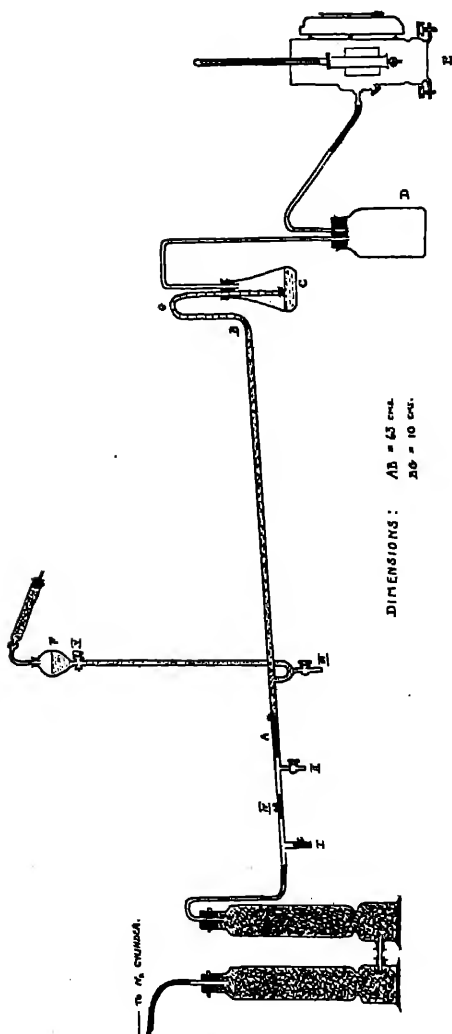


FIG. 1.

was allowed to bubble through potassium oleate solutions and the foam so created was collected in a flask and analysed for total potassium and fatty acid. The original and residual solutions were similarly analysed and the loss or gain of soap estimated. Positive adsorption was shown to take place.

Laing next employed a static method in which a foam was produced by constant agitation of the dilute sodium oleate solution in an atmosphere of purified nitrogen. The foam was freed from residual liquor by draining and both were analysed to show changes in concentration, the methods of electrical conduction, refractive index and complete chemical analysis being employed. In every case the foam gained in concentration at the expense of the bulk of the liquid, the composition of the films depending upon the composition of the original solution. These experiments showed that soap containing excess of fatty acid accumulates at the surface of the foam except from a solution containing slight excess of alkali.

The next experiments were carried out by H. C. Hillman with a slightly alkaline solution of Kahlbaum's sodium oleate. In this work the area of the films' surface was determined by estimating the number of bubbles and the total volume of the nitrogen used.

In three completed experiments he found that neutral sodium oleate was adsorbed; the number of equivalents per sq. cm. being 1.43, 1.50 and 1.42, respectively; that is, the area per oleate radical was 11.7, 11.1 and 11.8 Å for each group of 18 carbon atoms. These results are closely duplicated in the present work.

EXPERIMENTAL

APPARATUS AND MATERIALS

The sodium oleate solutions used in this investigation were prepared from pure sodium drippings and especially pure oleic acid made by the British Drug Houses according to directions supplied by Prof. A. Lapworth.³ Twentieth-normal sodium oleate solution was used throughout.

The apparatus was set up as shown in Figure 1. The nitrogen leaves the cylinder through a regulating valve at a small head of pressure and passes through the purifying towers which contain pumice, sodium hydroxide and pyrogallol. To ensure smooth bubbling the jet orifice (Fig. 2) must be ground smooth, cleaned and rigidly clamped. The solution tube *AB* (63 cm. long and 0.5 cm. in diameter) is fitted with exit taps. Tap 2 is used for the removal of any liquid running back through the

³ *Biol. J.*, 19, 7 (1925).

jet at the beginning of an experiment and tap 3 for the withdrawal of the entire solution after an experiment for analysis as residue. *AB* is sloped at an angle of 20° to the horizontal and terminates in a narrow

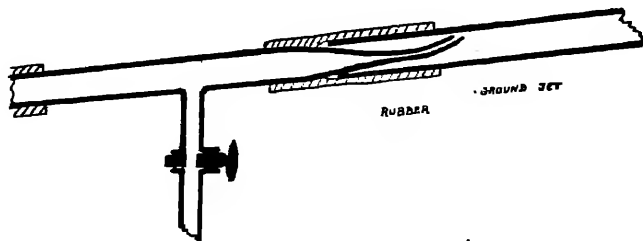


FIG. 2.

film delivery tube, *B*, which is vertical for 10 cm. to allow drainage and then curves through 180° and ends in a jet within the receiver *C*. *D* is a trap and *E* a flowmeter reading to 0.0001 cu. ft. for the measurement of the volume of nitrogen used in the experiment.

PROCEDURE

The apparatus is thoroughly cleaned, rinsed with soap solution and assembled for a run, an empty duplicate receiver being attached.

Screw clip 4 is closed, *I* opened, and nitrogen allowed to flow through the towers to expel air. The working solution from *F* is then allowed partly to fill the apparatus. The nitrogen regulator and tap 1 are closed and 4 opened. Tap 5 is now opened until the solution reaches the constant level point *B* when the flow is adjusted so that the solution level remains constant. The nitrogen flow is started and the size of bubble altered to the required dimensions by movement of the jet, which is then firmly clamped in position. When bubbling is constant the empty receiver is replaced by a weighed vessel containing a known weight of 0.1*N* HCl. The amount of this acid which is neutralised measures the total sodium carried over in the soap bubbles. The experiment is timed from this moment and lasts about 7 hours. At 30 minute intervals the rate of bubbling was determined by timing 100 bubbles and the total for the run calculated from the average number.

ANALYTICAL METHODS

The oleic acid in the receiver is separated either by filtration⁴ or by extraction with chloroform.⁵ Thereupon the number of equivalents of

⁴ McBain and Bowden *J. Chem. Soc.*, 123, 2417 (1923).

⁵ McBain and Quick, *J. Chem. Soc.*, 127, 1403 (1925).

oleic acid is estimated by titration with alcoholic sodium hydroxide. The latter method proved the most accurate.

CALCULATION OF RESULTS

The general equation⁶ used in the calculation of the amount of adsorbed material Γ in equivalents per sq. cm. is given by

$$\Gamma = \frac{W}{A} \left(c_t - \frac{c_o + c_r}{2} \right)$$

where W is the weight of the collapsed film in equivalents of sodium or oleate radicle per gram of solution, A is the surface area, c_t , c_o and c_r are respectively the concentration of foam, original solution and residue all expressed as equivalents per gram of solution.

All the terms in the equation are obtained from the experimental data. The surface area is calculated from the volume of the bubble since we know the total volume of gas passed and the number of bubbles. While passing through the solution the bubble is approximately spherical and if it is assumed that adsorption equilibrium is reached only after an appreciable time then the area perhaps should be calculated from the spheres. However, if adsorption is instantaneous the area of the film as it enters the film delivery tube is the true adsorbing area, and under such conditions the bubble is a right cylinder whose radius is that of the tube (r) and whose length (l) can be readily calculated from the volume. Hence surface area $= 2\pi r^2 + 2\pi r l$. The true adsorption may be taken as lying between the limits calculated for spherical and cylindrical films. The calculations are given for spheres and for cylinders for both sodium and oleate radicles. The detail of the experimental procedure is best illustrated by giving in full the data of an actual experiment:

<i>Data.</i>	Duration of experiment.....	6 hours
	Volume of nitrogen passed.....	2586 cc.
	Time for 100 bubbles.....	58.05 sec.
	Time of contact of N ₂ with solution.....	11.6 sec.
	Radius of film delivery tube.....	0.227 cm.
	Weight of foam collected.....	12.9131 gm.
	Total number of films.....	37210
	Volume of bubble.....	0.06949 cc.

Analysis. (Concentrations expressed as equivalents per gram solution $\times 10^{-3}$.)

	Original	Residue	Foam	Per Cent Increase	Ratio	Composition
Na.....	4.84	4.68	5.00	3.20	Ol/Na 1.655	NaOl.0.66HOI
Ol.....	4.83	4.59	5.10	5.29		

⁶ Fully deduced in McBain and Davies (*loc. cit.*).

Total surface area.

(1) as spheres (radius = 0.2551 cm.) = 30420 sq. cm.

(2) as cylinders (length 0.4293 cm.) = 34830 sq. cm.

Adsorption Γ calculated from above data.(a) From oleate analyses $\left(c_t - \frac{c_o + c_r}{2}\right) = 0.24 \times 10^{-3}$ equiv.Hence (1) $\Gamma = 1.019 \times 10^{-3}$
(2) $\Gamma = 0.8896 \times 10^{-3}$ equivalents per 1 sq. cm.Hence area per atom of Na (1) 16.19 Å
(2) 18.55 Å(b) From sodium analyses $\left(c_t - \frac{c_o + c_r}{2}\right) = 0.39 \times 10^{-3}$ equiv.(1) $\Gamma = 1.656 \times 10^{-3}$
(2) $\Gamma = 1.445 \times 10^{-3}$ equivalents per 1 sq. cm.Hence area per C_{25} group (1) 9.96 Å
(2) 11.42 Å

EXPERIMENTAL DATA

The results of the investigation are reported in Tables 1 to 6 inclusive. Table 1 shows the preliminary experiments of Mr. H. C. Hillman. Table 3 records the analytical data upon which the subsequent calculations are based.

TABLE 1. *Adsorption from Slightly Alkaline Sodium Oleate Solutions.*
(Concentrations as equivalents per gram solution $\times 10^{-3}$)

Original c_o		Foam c_f		Residue c_r		Adsorption $\Gamma \times 10^{-3}$ Equivs. per Sq. Cm. as NaOl	
Na	Ol	Na	Ol	Na	Ol	Spheres	Cylinders
5.02	4.92	4.86	4.77	5.24	5.10	1.639	1.427
5.36	5.34	5.20	5.13	5.78	5.69	1.747	1.503
5.36	5.34	5.19	5.13	5.65	5.65	1.620	1.415

TABLE 2. *The Area of Soap Solution per Molecule of Adsorbed Sodium Oleate.*

No. of Bubbles	Total Surface Area as Spheres	Area per Molecule $\times 10^{18}$ Sq. Cm.	Total Surface Area as Cylinders	Area per Molecule $\times 10^{18}$ Sq. Cm.
32550	14000	10.3	16100	11.68
45430	19900	9.7	22900	11.09
40840	19400	10.3	22200	11.78

(Radius of spheres 0.185, 0.187, 0.195; length of cylinders 0.289, 0.297, 0.335 cm.)

TABLE 3. Complete Chemical Analysis of Solutions of Sodium Oleate and the Foams Derived Therefrom.

(Concentrations as equivalents per gram of solution $\times 10^{-4}$.)

Original c_o		Residue c_r		Foam c_f		Per Cent Increase in Foam	
Na	Ol	Na	Ol	Na	Ol	Na	Ol
4.83	4.82	4.77	4.73	5.11	5.17	6.46	8.39
4.84	4.83	4.68	4.59	5.00	5.10	5.04	8.28
4.84	4.83	4.71	4.55	5.03	5.14	5.45	9.59
4.94	4.95	4.81	4.73	5.09	5.20	4.52	7.44
4.94	4.95	4.83	4.78	5.07	5.19	3.89	6.73
4.94	4.95	4.82	4.78	5.068	5.199	3.88	6.89

TABLE 4. The Amount of Adsorption on Spherical Bubbles and the Average Area of Surface per Atom of Sodium and per C_{18} Group.

No. of Bubbles	Radius of Sphere (Cm.)	Total Surface Area (Sq. Cm.)	$\Gamma \times 10^{-9}$	Area per Na Atom (Sq. Å)	$\Gamma \times 10^{-9}$	Area per C_{18} (Sq. Å)
			Sodium Equivs. per Sq. Cm.		C_{18} Equivs. per Sq. Cm.	
30630	0.206	16280	1.184	13.94	1.1529	10.79
37210	0.255	30420	1.019	16.19	1.656	9.96
31030	0.236	21790	1.161	14.21	2.010	8.21
26510	0.289	27740	0.892	18.50	1.494	11.04
38070	0.267	34000	0.683	24.16	1.182	13.96
34960	0.270	31230	0.723	22.82	1.280	12.89
Mean value.....				18.30		11.13

TABLE 5. The Amount of Adsorption on Cylindrical Bubbles and the Average Area of Surface per Atom of Sodium and per C_{18} Group.

No. of Bubbles	Length of Cylinder (Cm.)	Total Surface Area (Sq. Cm.)	$\Gamma \times 10^{-9}$	Area per Na Atoms (Sq. Å)	$\Gamma \times 10^{-9}$	Area per C_{18} (Sq. Å)
			Sodium Equivs. per Sq. Cm.		C_{18} Equivs. per Sq. Cm.	
30630	0.225	19740	0.9955	16.57	1.260	13.09
37210	0.429	34830	0.8896	18.55	1.445	11.42
31030	0.344	25240	1.002	16.47	1.735	9.51
26510	0.622	29450	0.840	19.64	1.407	11.73
38070	0.491	38940	0.596	27.68	1.032	15.99
34960	0.511	36930	0.611	27.00	1.083	15.23
Mean value.....				20.98		12.83

TABLE 6. The Composition of the Material Which Accumulates in the Foam from Neutral 0.05N Sodium Oleate.

Per Cent Increase	Per Cent Increase	Ratio	Composition
Na	Ol	Ol/Na	
6.46	8.39	1.299	NaOl.0.3HOl
5.04	8.28	1.64	NaOl.0.64HOl
5.45	9.59	1.72	NaOl.0.72HOl
4.52	7.44	1.65	NaOl.0.65HOl
3.89	6.73	1.73	NaOl.0.73HOl
3.88	6.89	1.77	NaOl.0.77HOl
Mean.....		1.70	NaOl.0.70HOl

DISCUSSION

On the surface of an $N/20$ solution of sodium oleate there is adsorbed one oleate radicle for each 11 sq. Å. The cross section of a hydrocarbon chain is known to be 20 sq. Å, hence the soap adsorbed in the surface of this dilute solution is more than can possibly be close-packed into a single monomolecular layer. The only other available quantitative data for soluble substances are those of McBain and Davies (*loc. cit.*) who obtained exactly the same result with, for example, aqueous solutions of amyl alcohol. It therefore appears probable that all these solutions even when far removed from saturation are covered by a complete monomolecular film of solute, and that there is an additional adsorption comparable in amount in the solution underneath the monomolecular film. The most plausible picture is that of chains of oriented molecules of solute, extending from numerous points on the monomolecular film deep into the surface layers of solution, as pictured for example in the diagrams of McBain and Davies. Such chains would be regarded as evanescent but extremely numerous continually growing out from the oriented molecules of the monomolecular film until broken up through thermal vibration in the liquid.

The adsorbed sodium oleate is partly hydrolysed. The hydrolysis corresponds to two-fifths of the total sodium being replaced by hydrogen. There is no reason for assuming that chains of free fatty acid would tend to form within the aqueous liquid, hence the hydrolysis is presumably that of the portions of monomolecular film exposed at any one moment to the aqueous solution beneath. In other words, we picture a hydrolytic equilibrium between the sodium ions of the electrical double layer and the hydrogen ions of the water. Some of the sodium ions of the exposed monomolecular film can wander away into the soap solution when their places are taken by hydrogen ion. The hydrolysis even of the exposed portions of the monomolecular film is by no means complete but is in equilibrium and it is completely suppressed by addition of even a slight excess of alkali to the soap solution. The surface conductivity will naturally depend greatly upon the position of this equilibrium. Measurements of conductivity of monomolecular films of insoluble fatty acids are being made at Stanford University by Mr. Peaker. The unpublished experiments of Darke,⁷ Dale, Salmon, Elford and Miss King in the Bristol Laboratories and Mr. Peaker in the Stanford Laboratories have proven the existence of such surface conductivity and have led to numerical values per sq. cm. of glass in contact with a wide range of concentrations of potassium chloride, which will shortly be published.

Reinold and Rücker⁸ proved that a soap film *in situ* possesses a

⁷ See *Trans. Farad. Soc.*, 16, 150 (1920); *Kolloid-Z.*, 28, 329 (1921).

⁸ *Phil. Trans.*, 184, 505 (1893).

greater conductivity than the bulk of the soap solution from which it is prepared, which is presumably due to this surface conductivity. Our picture would make it appear that they over-estimated the thickness of the different black films which they found to be 100-300 Å (that is, for a double-adsorbed layer), because they assumed that the material in the film did not have a greater refractive index than the bulk of the soap solution. They noticed significant changes in conductivity as the film became thinner, almost amounting to a discontinuity when the two mathematical surfaces approached within about 1000 Å of each other. This suggests a limit for the distance to which chains extend into the solution. The presence of chains to such depths would explain the many observations of the older physicists which pointed to an effective range of molecular action of about this magnitude. If the soap film becomes thin enough there is not room for the normal amount of chain and it might be expected that the composition of the film would therefore be affected which would again accord with the observations of Reinold and Rücker.⁹ Milner was of the opinion that the material which we assume to be present in the form of chains was extruded from the film as it thinned in the form of visible flakes but he took no precautions against decomposition by carbon dioxide. Reichenbacher¹⁰ expressed the opinion that in the black film the two adsorbed layers come in contact but this view of our experiments would not mean the whole of the adsorbed but merely the two monomolecular films. In the experiments of Perrin and Wells,¹¹ where it is assumed that the monomolecular layers are composed of oleic acid, solutions were subject to the action of carbon dioxide.

The most general result is the contradiction between the amounts of adsorption predicted by Gibbs and those observed, the latter being greater. As pointed out in the introduction the predicted adsorption of the soap is wrong not merely in magnitude but in sign. This is not due to the use of concentration instead of activity or potential because these likewise must increase with increase in concentration, leaving the sign of the prediction unchanged. Again, it is not due to any particular state of aggregation in the surface because an examination of the derivation of Gibbs or of Lewis and Randall shows that nothing whatsoever is postulated with regard to the state in the surface, the discussion being confined to the properties of the phases far away from the surface. The strict application of the Gibbs theorem does however require that all the components be taken into account, and the factor of hydrolysis introduces an extra component and therefore an extra term in the equation. This can be evaluated experimentally but it is hardly likely that it

⁹ See for example the calculation of Milner, *Phil. Mag.* (6), 13, 105 (1907).

¹⁰ *Kolloidchem. Beihefte*, 8, 139 (1916); and Freundlich, "Kapillarchemie," 1922, p. 1099.

¹¹ *Ann. Phys.*, 10, 160-84 (1918); and 16, 69-110 (1921).

can effect a reconciliation. As pointed out by McBain and Davies, the thermo-dynamic treatment has ignored the electrification that occurs at all surfaces. The only alternative explanation would be that all dynamic measurements of adsorption are in error.

SUMMARY

Contrary to the predictions of the Gibbs theorem, both in its popular and in its exact form, sodium oleate is positively adsorbed in the air-water interface. The amount adsorbed is nearly twice as great as can be packed into a monomolecular film. This parallels the findings of McBain and Davies with aqueous solutions of such substances as para-toluidine, camphor and amyl alcohol and supports their suggestion that the surface of a solution may be covered with a monomolecular film of adsorbed solute but may also exhibit a high concentration of solute in the neighborhood of the surface, probably in the form of chains of oriented molecules of solute extending from the monomolecular film deep into the surface. The adsorbed soap is in hydrolytic equilibrium with the solution but its hydrolysis is completely suppressed by a small excess of alkali.

*Department of Chemistry,
Stanford University,
California.*

THE ADSORPTION OF METHYLENE BLUE BY LEAD SULFATE

BY WILDER D. BANCROFT AND C. E. BARNETT

Paneth and Vorwerk¹ have studied the adsorption of Ponceau 2R by crystals of lead sulfate. When the surface has taken up all the dye that it will, they estimate that there is about one molecule of the dye to every eleven molecules of lead sulfate in the surface. If the dye molecules were cubical, they would cover only 31.3 per cent of the surface. On the assumption that they are rather flat parallelopipedons, they can be made to cover the surface completely with a layer one molecule thick. Conversely, if one assumes a layer one molecule thick with specified dimensions for the molecules, one can calculate the surface. Paneth has done that and has found a satisfactory agreement with the results obtained by other methods.

Paneth ignored the fact that the amount of a basic or acid dye taken up by wool, for instance, is a function of the pH, more of a basic dye being taken up with increasing pH, at least within certain limits, and less of an acid dye. This would seem to indicate a possible error in Paneth's work. On the other hand it was conceivable that the effect of pH appears only so long as the surface is not covered completely and that the maximum amount of dye which can be taken up by a fiber or a crystal may be independent of the pH.

Paneth and Radu² measured the surface of a sample of powdered diamond microscopically, and determined it also from the maximum amount of methylene blue adsorbed from an aqueous solution on the assumption that the surface is covered one molecule thick. Since the agreement was apparently satisfactory, they conclude that the same rule holds for the adsorption of dyes by other forms of carbon and they use the method to calculate the specific surfaces of various charcoals. This method has also been used by Rideal and Wright³ for the determination of the specific surface of a nitrogen charcoal.

Marc⁴ had studied the adsorption of dyes from aqueous solution by solid powders and had apparently shown that a given amount of a powdered solid can only take up a certain maximum amount of a dye. When this maximum amount of the dye has been adsorbed, a "saturated surface" of the adsorbent is formed. It was also shown that the order in which various dyes are adsorbed is a function of the crystallographic nature of the adsorbing surface. Marc made up his solutions from the dye and did not vary the pH knowingly, though he undoubtedly did actually. Consequently there is the same tacit assumption in his work

¹ *Z. physik. Chem.*, 101, 480 (1922).

² *Z. physik. Chem.*, 101, 488 (1922).

³ *J. Chem. Soc.*, 129, 1813 (1925).

⁴ *Z. physik. Chem.*, 73, 685 (1910); 75, 710 (1911); 81, 641 (1912).

that there was in Paneth's—that the maximum amount of dye adsorbed is independent of the pH.

It seemed desirable to check the assumptions involved. Experiments were first made to determine the adsorption of methylene blue by crystals of lead sulfate as one would ordinarily do it in studying adsorption—working with a constant and moderate amount of methylene blue and a varying pH. When it had been shown that the general behavior of methylene blue is the same with lead sulfate as with wool, experiments were made with varying concentrations of methylene blue in buffered solutions of practically constant pH, to see whether the maximum adsorption at pH = 4 differs from that at pH = 5.

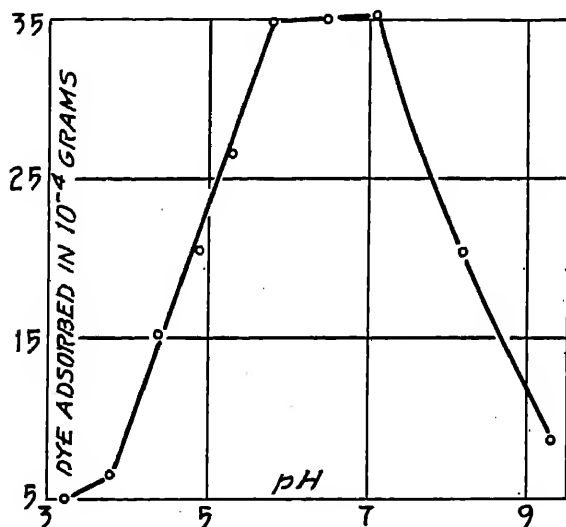


FIG. 1.

Following the example of Paneth and Vorwerk, lead sulfate was precipitated from a dilute solution of lead nitrate (fifteen grams per liter of distilled water) by addition of molecular sulfuric acid. The precipitate was washed by decantation, placed in a shaking machine, and shaken until a suspension was formed. Samples for use in the adsorption experiments were removed with a pipette. Two samples, which were analyzed, gave a concentration of 0.868 gram per 100 cc. of the suspension.

In preparing the lead sulfate the precipitate must not be dried because the agglomerated particles formed during the drying are difficult to break up even with constant shaking. Since the specific surface of the lead sulfate is constantly being increased due to this separation, it is almost impossible to obtain maximum values for the adsorption.

The amount of dye adsorbed in each case was determined by differ-

ence from the initial and final concentration. The analysis of the dye by titration with titanous chloride⁵ was found to be more satisfactory than the colorimetric determination.

In the first series of experiments the pH was varied from 3.2 to 9.3 by adding tenth-normal sodium hydroxide solution to the dye solution. The apparent hydrogen-ion concentration was determined by means of Biilmann's quinhydrone electrode. The dye solution contained 0.5932 gram of methylene blue in one liter of solution, 50 cc. of solution being used in each experiment.

The data are given in Table I and are reproduced graphically in Fig. 1.

TABLE I. *Effect of Varying pH on the Adsorption of Methylene Blue by Lead Sulfate.*

N/10 NaOH	pH	Dye Adsorbed per Gram PbSO ₄ in 10 ⁻⁴ Grams	N/10 NaOH cc.	pH	Dye Adsorbed per Gram PbSO ₄ in 10 ⁻⁴ Grams
0.0	3.2	5.0	7.0	5.8	34.5
0.5	3.9	6.1	8.5	6.5	35.0
1.0	4.3	15.0	10.0	7.1	35.4
3.0	4.9	21.2	15.0	8.2	20.8
5.0	5.3	28.8	20.0	9.3	12.2

With increasing pH there is an increase in the amount of methylene blue adsorbed up to about 7.3, after which there is a rapid decrease. In alkaline solutions the color base becomes colloidal and that accounts for the decrease in the adsorption. Curves of the same general type have been obtained in the Cornell laboratory for the adsorption of methylene blue by wool.

In the second set of experiments, solutions of ten different concentrations of methylene blue were buffered at pH values of 4 and of 5, 50 cc. of each solution being taken for the adsorption. The data are given in Table II and are shown graphically in Figure 2.

TABLE II. *Effect of Varying the Concentration on the Adsorption of Methylene Blue by Lead Sulfate.*

Initial Conc. Methylene Blue in 10 ⁻⁴ Grams per 50 cc.	Dye Adsorbed in 10 ⁻⁴ Grams per Gram PbSO ₄		Initial Conc. Methylene Blue in 10 ⁻⁴ Grams per 50 cc.	Dye Adsorbed in 10 ⁻⁴ Grams per Gram PbSO ₄	
	pH = 4.0	pH = 5.0		pH = 4.0	pH = 5.0
44.5	0.6	5.1	300.4	13.0	23.0
63.7	2.6	8.3	450.1	14.0	29.0
121.0	4.8	11.0	535.3	15.0	28.0
143.7	4.4	15.0	669.1	14.0	30.0
225.0	8.2	16.0	750.9	15.0	29.0

At maximum adsorption there is in round numbers twice as much methylene blue adsorbed by lead sulfate at pH = 5.0 as at pH = 4.0. If the data had been obtained at pH = 4.0 and pH = 6.0 or 7.0, the difference would doubtless have been much more striking. These figures are sufficient to show that the method is of no value for determining

⁵ Knecht, *Ber.*, 36, 1552 (1903); 40, 3819 (1907).

total surface and that the agreement found by Paneth between his microscopical and his adsorption experiments was either coincidence or a matter of skillful calculation.

There is an apparent contradiction between these results and those of Marc,⁶ because Marc reports that methylene blue is not adsorbed at all by lead sulfate. There are two reasons for this. Marc prepared his lead sulfate by precipitation from a solution of lead nitrate at the boiling-point. He then washed and dried the precipitate, a proceeding which would give a relatively small surface and a relatively low adsorbing power. The other reason is that Marc's analytical method was only accurate to about ten per cent and he wrote down zero if the adsorption by the lead sulfate was less than ten per cent of the initial concentra-

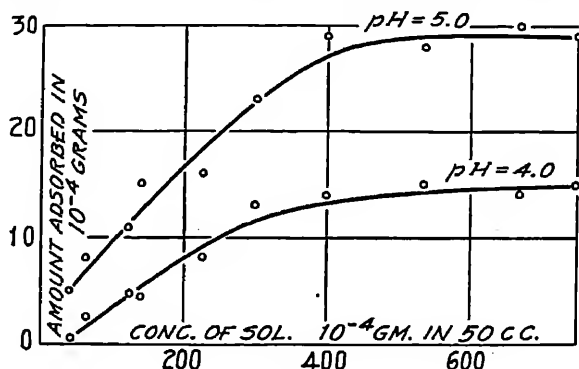


FIG. 2.

tion. Consequently at the hydrogen ion concentration of methylene blue saturated with lead sulfate, the amount of methylene blue adsorbed by a good one-gram sample of lead sulfate would be less than ten per cent of the initial concentration and would therefore have been within the limit of Marc's experimental error.

The general conclusions to be drawn are:—

1. For any concentration of dye the amount of adsorption by a powdered solid is a function of the apparent pH of the solution.
2. It is not possible at present to use the maximum adsorption of a dye by crystals as a measure of the total surface. Paneth's method is therefore not reliable in its present form.
3. Marc's statement that lead sulfate crystals do not adsorb methylene blue is in error. It is true, however, that the amount of adsorption comes within the limits of his relatively large experimental error.

Cornell University,
Ithaca, New York.

⁶ *Z. physik. Chem.*, 75, 710 (1911).

THE EFFECT OF TEMPERATURE ON THE COAGULATION OF COPPER COLLOIDAL SOLUTION

BY E. F. BURTON AND MRS. BEATRICE REID DEACON

The experiments outlined in this paper are a continuation of work on the so-called "coagulation temperature" of copper colloidal solution, a paper which has already been published.¹ It was found that, if samples of a copper colloid were raised to different high temperatures and maintained at those high temperatures for half an hour, there existed one temperature below which the samples were not coagulated and above which they were coagulated as a result of this heating. This temperature has been termed the "coagulation temperature" of the colloidal solution. It is, of course, dependent on the exact experimental conditions, and not strictly comparable with the term as used in colloidal literature. The fact that no ebullition took place in these experiments established one marked difference from previous work on coagulation temperature. The nature of the vessel containing the colloid is another factor which was found to influence coagulation at high temperatures. Impurities dissolve from glass which prove effective coagulants in the case of copper colloidal solutions; in this work a copper tube was used.

The experimental procedure was simple; a sample of colloid was sealed in a tube which was heated to any desired temperature for half an hour. Figure 1 shows the arrangement of the apparatus. The tube was placed in glycerin which has a boiling point of 290° C., and the glycerin was heated in an electric furnace. The furnace consisted of a cylindrical copper vessel, 19 cm. in height and 11 cm. in diameter, surrounded by a coil of wire which was insulated with asbestos. A copper lid fitted tightly over the top of the furnace. The end of the condenser for the glycerin and a thermometer of range 0° to 200° C. were inserted in a cork fitting into an opening in the center of the lid. The furnace was connected to the 110 line in series with a Zenith variable-resistance coil and an ammeter.

A cylindrical copper tube, 10 cm. long and 1.5 cm. in diameter, was used to contain the copper colloid. After 12 cc. of the solution were

¹ Deacon and Burton, *J. Phys. Chem.*, 32, 425 (1928).

pipetted into the tube, the resistance of the sample was measured using two platinum electrodes; the electrodes were made so that they could be inserted directly into the copper tube. The tube containing colloid was then dropped onto glass wool in the bottom of a thick-walled glass tube. Water was introduced into the glass tube up to about 2 cm. from the

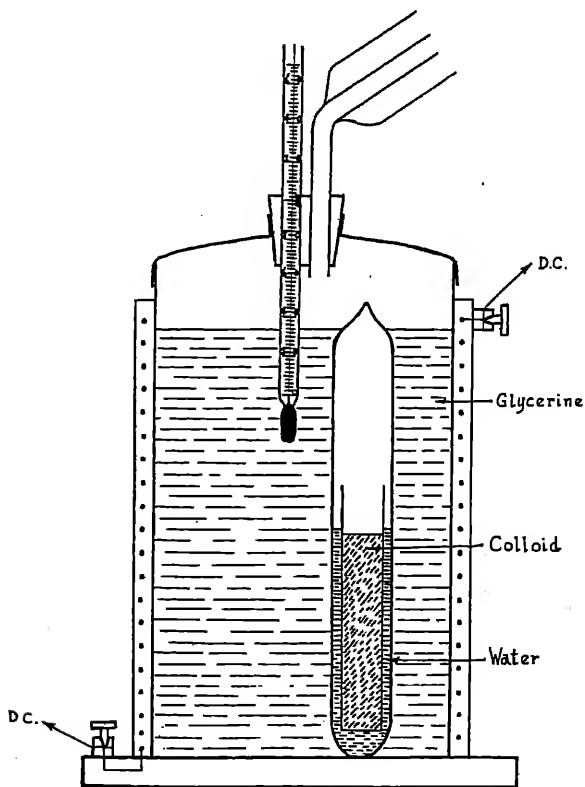


FIG. 1.

top of the copper tube. The glass tube was then sealed off and placed in the electrically heated glycerin bath.

About half an hour was taken to raise the temperature of the bath to the desired point, using a current of 5 amperes. By adjustment of the rheostat the glycerin was maintained at this temperature for one half hour. At the end of this time the tube was removed and allowed to stand at room temperature for four hours. Then the glass tube was opened,

and the copper tube removed; the resistance was again measured in order to check the introduction of impurities. The colloid was then poured into a clean test-tube in order to observe whether it had coagulated or not.

By this method it has previously been determined that for any given copper colloid, existing in a state of equilibrium, there is a definite temperature at which coagulation takes place automatically. The qualification "for a given time of heating" should be added to this statement in view of subsequent work detailed below. It has been shown that this coagulation temperature decreases as the colloid ages, that is, during the progress of slow coagulation. Experiments with samples of pure copper colloidal solution to which increasing amounts of *potassium chloride* were added indicated that the coagulation temperature decreased with increasing concentration of this electrolyte. The relation between the coagulation temperature and the concentration of electrolyte was approximately linear.

Experiments have since been carried out with samples of a stock solution of pure copper colloid to which were added increasing amounts of *potassium sulfate*. The same procedure as outlined above was followed; the temperature in each case was maintained constant for half an hour.

In these experiments the test-tubes into which the colloid was poured were first carefully cleaned and steamed, and afterwards corked. All the samples heated were kept in such test-tubes and comparative observations made of subsequent coagulation. It was found that all the samples which had been heated coagulated within a period of twelve weeks. Hence the state at the end of an arbitrary period of twenty-four hours is given as well as the state on opening the tube. The coagulation temperature was arrived at from these observations. This lends an artificiality to the term "coagulation temperature," as used in this paper, but in no way invalidates the significance of the results.

Table I gives the results. Each solution is defined by the concentration of added electrolyte. Two critical temperatures for each solution are shown from which the coagulation temperature may be approximately deduced. The state of the sample is indicated for each temperature:

- (1) just after the tube was opened (four hours after heating), and
- (2) after a period of twenty-four hours had elapsed.

The relation between concentration of added electrolyte and coagulation temperature in these experiments is not the simple linear one indicated by the previous results. The ion effective in coagulation in the

TABLE I.

Concentration of Electrolyte	Temperature Maintained Degrees C.	State		Coagulation Temperature Degrees C.
		4 Hours After Heating	24 Hours After Heating	
0	185-186	no coag.	slight coag.	187
	187-188	coag.	
$3 \times 10^{-7}N$	165-166	no coag.	partial coag.	167
	168-169	coag.	
$6 \times 10^{-7}N$	167-168	no coag.	no coag.	169
	169-170	coag.	
$9 \times 10^{-7}N$	165-166	no coag.	no coag.	168
	167-168	no coag.	partial coag.	
$12 \times 10^{-7}N$	173-174	no coag.	slight	175
	175-176	coag.	

previous experiments was the negatively charged univalent chlorine ion. The effective ion in these experiments was the negatively charged divalent sulfate ion. The results suggest the zonal character of the influence of electrolytes on colloidal solutions which is a well-known phenomenon where ions of higher valencies are concerned. The hypothesis of the existence of a zone of instability when the first traces of potassium sulfate are added, followed by a zone of increasing stability with further additions of electrolyte, presents the most plausible explanation of these results. It must be remembered that the amounts of electrolyte involved are extremely small.

Subsequent observations of the samples heated showed a consistent progress in coagulation. Comparative observations were made at intervals of a week. With the colloid having a normality 12×10^{-7} potassium sulfate, for example, a sample heated to 169° – 170° C. on February 16 was uncoagulated when the tube was opened. Slight coagulation was first observed on April 2 and complete coagulation on April 16. A sample of the same solution heated to 173° – 174° C. on February 17 showed slight coagulation on February 18 and complete coagulation on March 12. For each solution similar effects were observed; those samples which were heated to higher temperatures coagulated in a shorter time than those heated to lower temperatures.

The evidence points to the fact that the effect of heating is a speeding up of the process of coagulation. This is connected with the question

of whether all sols are undergoing slow coagulation at ordinary temperatures. A series of experiments was carried on with this question in view.

The coagulation temperature of the pure stock copper colloid had been determined by the method described above. It was emphasized that the high temperatures were maintained for half an hour in every instance. Now using the same pure stock solutions the time of heating was increased to 45, 60, 75, 90 and 105 minutes, respectively. The coagulation temperature corresponding to each time of heating was determined. Not enough of the solution was left to determine the coagulation temperature for a longer time. One sample, however, was heated for four hours to 81°–82° C. This sample was uncoagulated when the tube was opened, four hours after heating, and was still uncoagulated twenty-four hours after heating. As in the previous experiments every sample heated eventually coagulated. The sample heated for four hours coagulated within six days after heating.

The results are contained in Table II.

TABLE II.

Time of Heating (minutes)	Temperature Maintained (°C.)	State		Coagulation Temperature (°C.)
		4 Hours After Heating	24 Hours After Heating	
30	185-186	no coag.	slight coag.	187
	187-188	coag.	
45	159-160	no coag.	no coag.	161
	161-162	coag.	
60	143-144	no coag.	no coag.	145
	145-146	coag.	
75	113-114	no coag.	partial coag.	115
	115-116	coag.	
90	105-106	no coag.	no coag.	107
	107-108	coag.	
105	99-100	no coag.	no coag.	101
	101-102	coag.	
240	81-82	no coag.	no coag.	

The relation between the temperature of coagulation and the time of heating may be more clearly seen on the graph where coagulation

temperatures as ordinates are plotted against times of heating as abscissas. The curve apparently tends to become asymptotic with the axis of time. The view that this is the case received support from the final

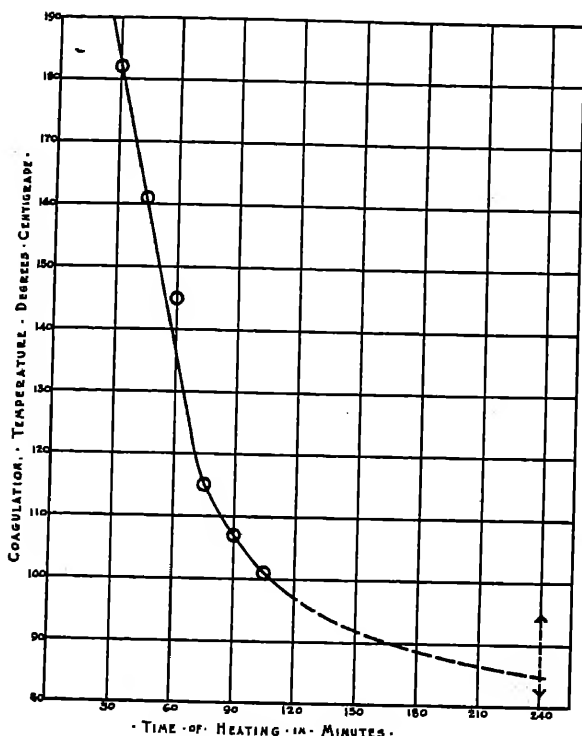


FIG. 2.

result where the colloid remained uncoagulated after four hours' heating to 81°–82° C. If this is so then at room temperature such a colloidal solution might be expected to remain stable for an infinite time.

*Department of Physics,
University of Toronto,
Toronto, Canada.*

THE STRUCTURE OF SOFTWOODS AS REVEALED BY DYNAMIC PHYSICAL METHODS

By ALFRED J. STAMM¹

Practically all of the available information on the structure of wood has been obtained by microscopical examination. Unfortunately, such studies have at least two major limitations. The first one is the limit of the resolving power of the microscope, which makes it impossible to observe structure finer than 0.1μ and to obtain any detailed information on even coarser structure. For this reason very little is known about the pit mechanism, the thinned portions of the cell wall that serve as a means of communication from lumen (fiber cavity) to lumen.² The capillary nature of the pit membranes remains quite unknown, though it is true that Bailey³ has shown in a qualitative way for seasoned wood that there is a continuous capillary communication from lumen to lumen through the pits even for colloidal India ink.

The second limitation of microscopical studies of wood structure is the difficulty involved in collecting statistical data on average dimensions of the various structural elements, such as tracheid (fiber) lengths and lumen diameters. An exceedingly large number of measurements have to be made in such cases in order to obtain fair averages. The time consumed in a study of this nature has greatly limited the amount of such information available.

Because of the limitations of microscopical methods, the dynamic physical methods considered in this paper were developed and applied. Four distinct methods of study have been used, namely, electroendosmotic flow, hydrostatic flow with the application of Poiseuille's law, the overcoming of the surface tension of liquids in wood capillaries by means of gas pressure, and the permeability of the sections to colloidal solutions containing particles of known size. The first of these was described in an earlier monograph² and all four have been described in detail in a series of three papers to appear in print soon.

¹ Associate Chemist in Forest Products, U. S. Forest Products Laboratory, Madison, Wis.

² Stamm, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 246.

³ *Forest Quart.*, 11, No. 1, 12 (1913); *Am. Ry. Eng. Assoc. Bull.* 16, 835 (1915).

ELECTROENDOSMOTIC FLOW

The determination of the velocity of electroendosmosis through wood sections, after having obtained the contact potential between the wood and water,² makes it possible to determine in turn the total effective capillary cross section q in sq. cm.

$$q = (300)^2 \frac{4\pi\eta V}{ED\zeta} \quad [1]$$

where V is the velocity of electroendosmotic flow in cc. per second, η the absolute viscosity of the liquid, E the potential gradient across the section in volts per cm., D the dielectric constant of the liquid, and ζ the contact potential between the adsorbed surface layer of the liquid and the bulk liquid expressed in volts.

The percentage total effective capillary cross sections of sections of several different softwoods cut in each of the three different structural directions were determined (100 q divided by the total cross section). The percentage total effective capillary cross section of thin transverse sections, most of the tracheids (fibers) of which are cut across twice, is quite large, approaching the total cross section of the open cell cavities, the value of which can be approximately calculated from the virtual density of the wood as a body, d , and the real density of the wood substance, d_o :

$$q_{\max} = \left(1 - \frac{d}{d_o}\right) \quad [2]$$

The percentage total effective capillary cross section of the tangential sections corresponds very closely to the internal ray cell cross section as determined microscopically. Though the ray cells furnish the chief capillary communication through these sections in a radial direction, communication through the few pits on the tangential faces of the tracheids has some effect. The percentage total effective capillary cross section of the radial sections is quite small and very likely it is made up almost entirely of communication through pits on the radial faces of the tracheids. The effective capillary cross section of the thin transverse sections is 10 to 100 times as great per unit surface area as that of the tangential sections and the latter in turn is 3 to 10 times as great as that of the radial sections.

EFFECT OF THICKNESS OF TRANSVERSE SECTIONS

Further structural information has been obtained by means of extended electroendosmotic flow studies. The velocity of electroendosmosis was determined over a large range of thicknesses of transverse sections. The results for two softwoods free from resin ducts are shown graph-

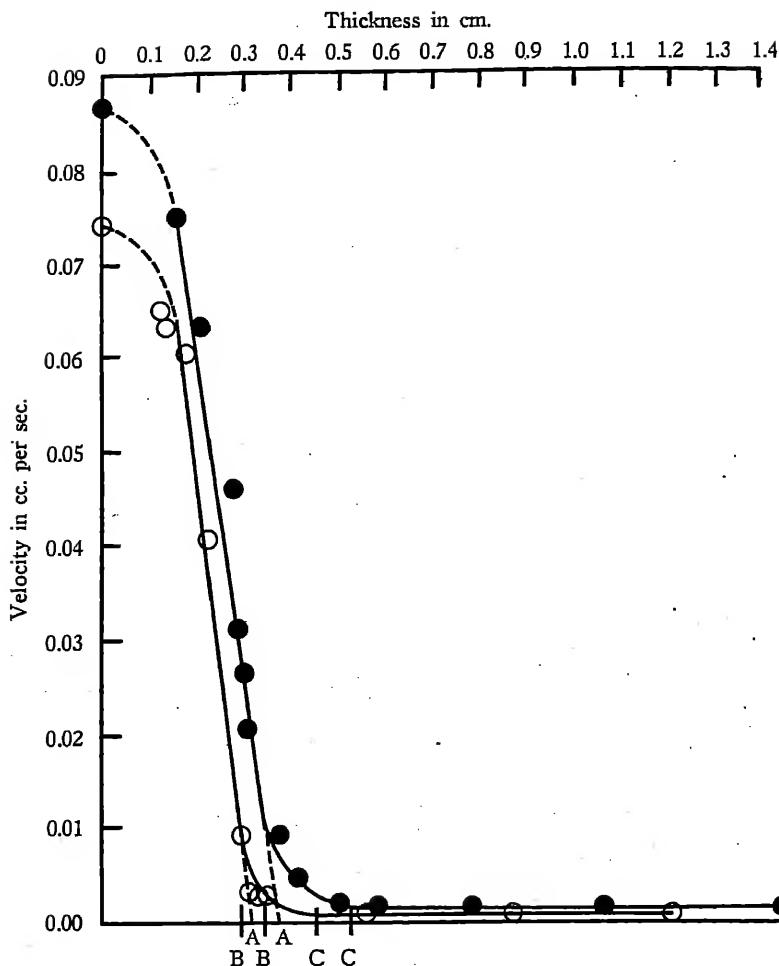


FIG. 1.—Change in Velocity of Electroendosmose with Thickness of Transverse Wood Sections.

○ Alaska Cedar, ● Western Red Cedar.
A: Average Tracheid Length.

B: Minimum Tracheid Length.
C: Maximum Tracheid Length.

ically in Figure 1. The velocity for the hypothetical zero thickness^{*} was calculated as for a section having the ideal void area, determined from

^{*} A dotted line has been used to connect the experimental points with this calculated point. Since the nature of the curve at this end has no bearing upon the conclusions drawn and the calculations made therefrom, the possible reasons for the deviation of this part of the curve from the elemental curve to follow will be omitted here.

the density of the wood, with E assumed constant. The rate of electro-endosmosis decreases rapidly for an increase of the thickness of the sections up to a value that is about the average tracheid length. Beyond this point it decreases more slowly, and finally becomes constant for a thickness greater than that which may be taken to represent the maximum tracheid length.

ELEMENTAL CURVE

A brief consideration of the nature of these curves seems necessary to bring out their full significance. To simplify the case, let us first suppose that wood is made up of uniform closed tubes of a given length, with no voids between adjoining outer surfaces, and that the position of these tubes, with respect to one another, in a longitudinal direction is perfectly arbitrary except that the arrangement is statistically uniform for as large a number of tubes as there are tracheids in an experimental section of wood (about one million). The proportion of tubes that have one end falling within any section cut across a bundle of them will thus vary directly as the thickness of the section, a straight-line relationship, up to the limit of a tube length. The effective open cross section will also vary directly from the total open cross section of the tubes, in the case of infinitesimally thin sections, to zero in the case of sections having a thickness equal to the tube length. When the tubes have a length different from that first assumed, a similar straight-line relationship will hold between the effective open cross section and the thickness of the section, but the slope of the line will be different. All such lines will have a common point for zero thickness of section, but the terminal point of each, which corresponds to the tube length, will be different. The dotted lines (2, 3, and 4) in Figure 2 represent the fractional change in open tube cross section with changes in thickness of the section for tube lengths of two, three, and four units.

If equal numbers of each of these three lengths of tubes are mixed in a single bundle of the same size as those assumed in Figure 2, the solid compound line D will result, the ordinates of which represent average values of the open capillary cross section corresponding to the respective section thicknesses of the bundle of mixed lengths.

In any softwood the tracheid lengths vary between definite limits. The deviations from the most probable length will presumably follow a form of Gaussian distribution law, and in most cases they will be only slight. These two conditions tend to cause the line representing the proportional change in open capillary cross section with respect to thickness of wood section to curve off gradually to the right, as tracheid lengths are exceeded by the thickness, in a manner dependent upon the nature

of the distribution of tracheid lengths, rather than to take the form of a series of broken lines like that of Figure 2.

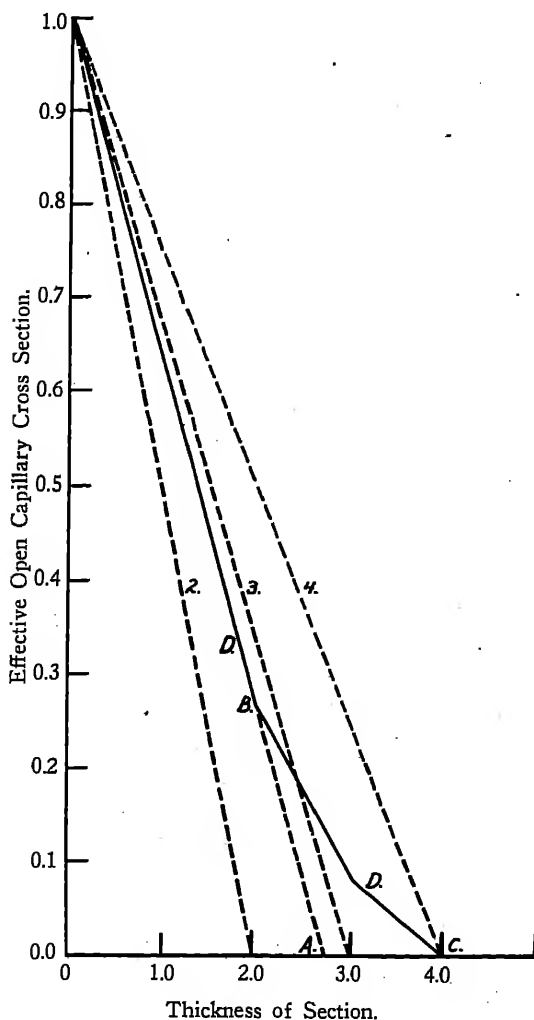


FIG. 2.—Change in Theoretical Effective Open Capillary Cross Section with Thickness of Section.

- A: Approximate Average Tube Length.
- B: Minimum Tube Length.
- C: Maximum Tube Length.

TRACHEID LENGTHS

The point (*B* in the elemental curve represented in Figure 2) where a typical curve such as one of those in Figure 1 starts to bend to the right corresponds to the minimum tracheid length. The bending is due to the gradual elimination of the shorter tracheids as effective open capillaries. The point *C* of Figure 2, where the curve intersects the axis of abscissae or, as in the case of an actual wood section, becomes parallel to it, corresponds to the maximum tracheid length. The point *A* of the same figure, where the continuation of the straight line through *B* intersects the axis of abscissae (or intersects the continuation of the limiting horizontal line in the case of actual wood), will approach the point corresponding to the average tracheid length as the deviation of the individual tracheid lengths from the mean becomes smaller and smaller. The deviation from the average tracheid length in the experimental wood sections may safely be assumed to be much less than in the hypothetical case because of the smaller mean deviation in the distribution of tracheid lengths, thus making *A* approximate the actual average tracheid length.

TABLE I.—*Tracheid Lengths in Centimeters as Obtained by Direct Measurement and by Three Different Dynamic Physical Methods.*

Species	Number Measured	Direct Microscopical Measurements			By Electroendosmotic Flow			By Over-static Surface Flow Tension	
		Min.	Ave.	Max.	Min.	Ave.	Max.	Max.	Max.
Western red cedar	0.31	0.38	0.45	0.33	0.38	0.53	0.55	0.56
Alaska cedar31	.35	.46	.45	.45
Sitka spruce	200	.22	.37	.49	.32	.38	.5253
Western yellow pine	100	.25	.33	.40	.30	.35	.5755
Douglas fir (Rocky Mountain type)	455044	.76	.32	.38	.5049

Table I gives some microscopically determined values of tracheid lengths previously collected by the Forest Products Laboratory and the corresponding values obtained from electroendosmotic-flow graphs, as well as some maximum tracheid-length data secured by other dynamic physical methods to be considered later. The agreement is satisfactory, especially in consideration of the fact that the direct-measurement values were obtained from entirely different specimens.

TOTAL CONTINUOUS EFFECTIVE CAPILLARY-COMMUNICATION CROSS SECTION

The elemental curve takes no account of a slight capillary communication from tube to tube, which corresponds to the continuous pit

communication in the case of actual wood. Superimposing this effect upon that already considered accounts very nicely for the deviation of the actual curves from the hypothetical, beyond the maximum tracheid length. The experimental curves become parallel to the horizontal axis beyond this point, showing that there is a continuous and uniform residual open cross section for each species of wood tested. This effective capillary cross section can be made up only of pit openings between tracheids, resin ducts, and open area caused by checks, etc. Microscopical examination of the thick sections showed the absence of appreciable checking for all of the species here listed. In the case of Alaska cedar and western red cedar, resin ducts are known to be entirely absent. Hence, their residual open sectional areas, shown in Table II, must represent those of the pit openings alone.

TABLE II.—Continuous Effective Open Area of Transverse Sections.

Species	V Velocity of Electroendos- mosis 30°C. cc./sec.	q (in percentage of total membrane area)	Resin Duct Cross Section (in percentage of total membrane area)	Corrected Percentage Pit Communica- tion Cross Section
Western red cedar	0.00053	0.52	none	0.52
Alaska cedar00035	.35	none	.35
Sitka spruce00081	.78	0.04	.74
Western yellow pine...	.00129	1.30	0.54	.76
Douglas fir (Rocky Mountain type)00033	.33	0.03	.30

Effective pit communication areas for the other species were obtained by subtracting the values for the microscopically determined resin-duct sectional area from the total continuous open cross section. It is of interest to note that the order of decreasing values of the percentage pit-communication cross section for the different species listed is the same as the order of increasing difficulty of impregnation with preservatives.

HYDROSTATIC FLOW

In the case of flow of liquids under hydrostatic pressure, the velocity of flow is dependent not only upon the total effective capillary cross section but also upon the actual effective bores of the individual capillaries making up the system. By combining the data from the electroendosmotic and the hydrostatic flow studies, it becomes possible to calculate approximately the actual size of the capillaries and to draw conclusions regarding the structure that determines the effective continuous capillary cross section. The same air-dry softwood membranes

used in the previous electroendosmosis experiments were used in the hydrostatic investigation so that the effective capillary diameters could be determined.

POISEUILLE'S LAW

The passage of liquids under external pressure through a smooth capillary tube of circular cross section for conditions of stream-line flow may be quantitatively expressed by Poiseuille's law,

$$V = \frac{\pi r^4 P}{8\eta l} = \frac{Ar^2 P}{8\eta l} \quad [3]$$

in which V represents the rate of flow, r the capillary radius, l the capillary length, P the applied pressure, η the absolute viscosity, and A the cross sectional capillary area, all in c.g.s. units. The equation has been experimentally verified by Osborn Reynolds⁵ for velocities below the critical value. High velocity of flow, large radius of the capillary, and short length, all tend to cause turbulent flow through the tube, a condition that results in deviation from the relationship of this law. The turbulence factor and the manner in which its effect has been minimized in the hydrostatic investigation will be considered later.

In the case of a bundle of N capillaries having a total effective capillary cross section q and a mean effective individual radius r , which more strictly speaking is the fourth root of the average of the fourth powers of the radii,

$$V = \frac{N\pi r^4 P}{8\eta l} = \frac{qr^2 P}{8\eta l} \quad [4]$$

If the bundle is made up of a statistically large number of tubes with deviations in cross section from the most probable value following a form of the Gaussian distribution law and with most of the cross sections deviating but slightly from the most probable value, r will not differ appreciably from the true average radius. This is the case for the open lumen capillaries of thin transverse sections of softwoods. It thus becomes possible to determine the approximate mean effective lumen cross sections and the corresponding diameters for the lumina, open at both ends, that make up a thin transverse section, by combining the hydrostatic data with the values of q previously determined.

PREVIOUS APPLICATIONS OF POISEUILLE'S LAW

Previous applications of Poiseuille's law for the determination of the dimensions of the capillary openings in natural and in artificial membranes are rather limited. This situation comes from the fact that

⁵ *Trans. Roy. Soc. (London)*, 3, 935 (1883).

neither N , the number of capillaries making up the membranes, nor q , the total effective capillary cross section, is readily obtainable. It is true that the average total capillary cross section in any plane through a membrane parallel to its faces can be calculated as in equation [2]. The result, however, may differ considerably from the effective value, which should be used in these calculations.

Gueront,⁶ making the simplifying assumption that the capillaries are prismatic tubes extending entirely through the membrane, was able to calculate the pore sizes of several natural and artificial membranes. Bigelow⁷ showed the applicability of Poiseuille's law to the capillary structure of several different membranes by proving that V is practically proportional to P over a large range of pressures (1 to 80 cm. of mercury) and is inversely proportional to the variation of η caused by change of temperature. He was unable to obtain actual values of the capillary dimensions, however, because q and N were unknown. Ducleaux and Errera,⁸ using the method of Gueront, obtained pore diameters for cellulose acetate membranes. Hitchcock⁹ obtained pore diameters using average pore cross sections as determined by density and electrical conductivity methods. Calculated values of these diameters varied directly as the thickness of the membranes, indicating that the effective pore diameter is a function not only of the size of the pore but also of the length of the path of flow. Bartell¹⁰ has done considerable work on pore dimensions using both Poiseuille's law and Jurin's method (measuring the pressure required to overcome surface tension in the capillaries). In the former case only relative values were obtained. Some recent, as yet unpublished, experiments of Bartell using packed carbon and silica membranes show that actual values of the pore diameters can be obtained by means of Poiseuille's law and that the values agree satisfactorily with values obtained by Jurin's method.

None of these investigators has employed the direct electroendosmotic method of determining the total effective capillary cross section which, in combination with the hydrostatic-flow method, was used in the present investigation. Gueront's assumption that the capillaries extend entirely through the membrane would have given accurate results for the pore diameters of transverse wood sections only in the case of infinitesimally thin sections in which none of the tracheids terminate in the section, and the deviations would have increased greatly with an increase in thickness. When the *effective* capillary cross section rather than the *average* is used, however, no assumptions need be made or

⁶ *Compt. rend.*, 75, 1807 (1872).

⁷ *J. Am. Chem. Soc.*, 29, 1675 (1907).

⁸ *Rev. gén. colloïdes*, 2, 130 (1924); 3, 97 (1925).

⁹ *J. Gen. Physiol.*, 9, 755 (1926).

¹⁰ *J. Phys. Chem.*, 16, 318 (1912); 27, 252 (1923).

corrections introduced for type of structure, since the effective capillary cross section itself takes into account the nature of the capillary systems.

DIFFERENTIAL METHOD AND APPARATUS

Instead of determining the volume of discharge of water from the capillary system per unit of time when subjected to definite head, with the system held at a constant temperature so that η is both determinable and constant, a differential method, which involves several special features, was used. If the membrane under test and a calibrated capillary tube are connected in series, with the following relations holding:

$$V_1 = \frac{q_1 r_1^2 P_1}{8\eta l_1} \quad [5] \text{ for a wood membrane}$$

$$V_2 = \frac{\pi r_2^4 P_2}{8\eta l_2} \quad [6] \text{ for a calibrated standard capillary tube}$$

then, since

$$V_1 = V_2 \quad [7]$$

because of the nature of the system

$$\frac{\pi r_2^4 P_2}{l_2} = \frac{q_1 r_1^2 P_1}{l_1} \quad [8]$$

solving for r_1

$$r_1 = \sqrt{\frac{\pi r_2^4 l_1 P_2}{q_1 l_2 P_1}} \quad [9]$$

This method of making the measurements, besides the obvious advantage of not requiring the system to be placed under thermostatic control, has the important advantage of making the attainment of equilibrium conditions easily ascertainable. Instead of carrying out a series of measurements of V for each set of experimental conditions, it merely is necessary to observe that the ratio P_2/P_1 has become constant.

The differential apparatus used for the hydrostatic flow studies (see Fig. 3) is similar in principle to that of Emanuelli¹¹ for determining the gas permeability of paper. It consists essentially of a large-bore glass tube divided into three compartments by the wood membrane M and the standard capillary tube C ; the former is clamped between brass flanges using pure gum rubber gaskets, and the latter is held by the rubber stopper R . Mercury manometers P_1 and P_2 indicate the pressure drop between compartments I and II, and II and III, respectively. A is the intake tube and B is the overflow. After fastening the wood membrane in place, the apparatus is filled with distilled water through tubes S and S' , care being taken to displace all air from the manometer tubes and connections. A constant hydrostatic head of water is applied at A , and when the pressure drops through the standard

¹¹ *Paper Trade J.*, 85, No. 10 (1927).

capillary and the membrane come to equilibrium, readings on the manometer tubes are recorded. Reversing the connections so that the hydrostatic head is applied at *B*, on the standard capillary side rather than on the membrane side, has no effect on the equilibrium pressure-drop ratio.

The effective area of cross section of the wood sections used in the

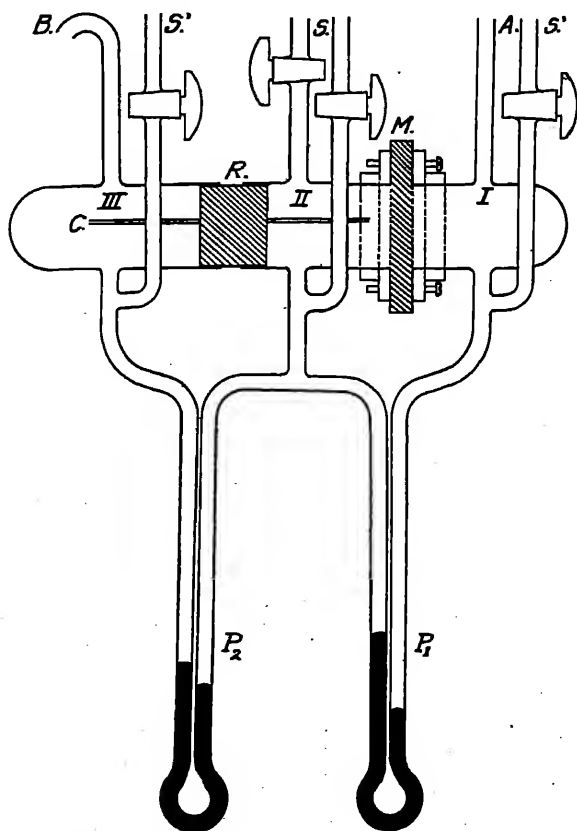


FIG. 3.—Apparatus for Determining the Pressure Permeability to Liquids of the Capillary Structure of Wood.

experiments was 2.08 sq. cm. The radii of the several standard capillary tubes ranged from 0.01102 to 0.03681 cm. and the lengths from 15 to 20 cm. The bores of these tubes were determined by the capillary-rise method, employing both benzene and water; tubes showing a maximum bore deviation of more than 0.5 per cent were rejected. The validity of Poiseuille's law for these standard capillaries was then checked by

direct trial measurements, using only compartments II and III and applying the hydrostatic pressure at S. The viscosity of the water was determined with the aid of a temperature-viscosity curve from the temperature of the efflux water, and the rate of flow from the weight of the efflux water and the time involved.

CORRECTION FOR TURBULENCE

The observed velocity through the tube employed was in all cases slightly less than the calculated value because of turbulence at the tube ends. The deviation decreased in practically a linear manner, over the range investigated, with a decrease in the applied head, and became negligible for pressures less than 0.2 cm. of mercury. Similarly, in using the differential method, a slight change in the pressure-drop ratio through the standard capillary and through the membrane, P_2/P_1 , resulted from a change in total applied pressure. This change in ratio comes from the fact that both P_2 and P_1 deviate from strict proportionality with the total applied pressure, because of loss of head, caused by turbulence, in each of the capillary systems. Although the change in loss of head caused by turbulence in one capillary system may differ from that in the other as the total applied pressure varies, in each case the total amount of such loss will approach zero as the total applied pressure approaches zero. Thus, if the ratio P_2/P_1 is plotted for a series of total applied pressures, the limiting value of P_2/P_1 obtained by extrapolating the curve to an infinitesimal value of applied pressure gives the condition for negligible turbulence effects. The graphical means of correcting for turbulence furnished by such a curve, which are described more fully in another paper, were used in all of the hydrostatic-flow experiments to be described here.

MEASUREMENT ON THIN TRANSVERSE SECTIONS

Measurements following the differential hydrostatic flow method were first made on thin transverse sections of different softwoods that were considerably less than a tracheid length in thickness. For these sections P_2/P_1 decreased about 20 per cent while the total applied pressure increased from a few tenths of a centimeter to five centimeters of mercury. Since the relationship between the ratio and the total pressure is approximately a straight-line one, the extrapolated values can be readily determined. The slope of the line indicates that P_1 increases much more rapidly than P_2 with an increase in total applied pressure. At higher pressures the deviation from Poiseuille's law, presumably caused by the combined turbulence factors, is greater for wood membranes than for the standard capillary tube. Table III gives the limiting pressure-drop ratios P_2/P_1 , the effective open cross section q

TABLE III.—Average Lumen Diameters Determined by the Application of Poiseuille's Law and by Direct Measurement.

Effective Membrane Surface = 2.08 cm., $l_2 = 16.02$ cm., $r_2 = 0.03681$ cm.

Species	Density of Wood	Thickness of Wood Section l_1 Cm.	$\frac{P_2}{P_1}$	q (in per- centage of total membrane area)	Average Diameter of Lumen	
					Poi- seuille's Law μ	Direct Measure- ment μ
Western red cedar..	0.290	0.165	29.5	76.7	21.0	27.6
Do.	Do.	.170	29.0	76.2	21.2	
Do.	Do.	.190	24.0	75.3	20.5	
Do.	Do.	.215	21.2	73.5	20.7	
Do.	Do.	.231	19.8	72.3	20.9	
Do.	Do.	.236	18.5	71.0	20.6	
Do.	Do.	.249	17.5	70.0	20.8	
Do.	Do.	.272	11.6	53.7	20.2	
Do.	Do.	* 0.157	26.7	77.7	19.3	
Do.	Do.	* .195	22.0	76.2	19.7	
Do.	Do.	* .211	18.0	74.7	18.8	
Do.	Do.	† .218	20.0	74.3	20.2	
Alaska cedar	0.442	0.145	20.7	61.1	18.4	23.1
Do.	Do.	.175	16.0	58.2	18.2	
Do.	Do.	.188	14.5	56.7	18.2	
Do.	Do.	.200	14.0	54.3	18.9	
Sitka spruce	0.304	0.167	31.0	69.2	22.8	26.3
Do.	Do.	.173	28.4	68.2	22.3	
Do.	Do.	.211	24.0	63.4	23.5	
Do.	Do.	.267	12.7	48.0	22.1	
Western yellow pine	0.410	0.114	28.5	65.3	18.6	23.0
Do.	Do.	.157	19.0	61.7	18.3	
Do.	Do.	.165	20.0	61.2	19.3	
Do.	Do.	.183	17.0	57.8	19.3	
Do.	Do.	.244	10.4	44.3	19.9	
Do.	Do.	.269	5.6	28.1	19.3	
Douglas fir (Rocky Mountain type) ..	0.526	0.130	16.0	55.9	16.1	20.6
Do.	Do.	.175	15.0	52.0	18.7	
Do.	Do.	.236	9.0	43.2	18.5	

* 95 per cent alcohol used instead of water.

† Benzene used instead of water.

expressed in per cent of the total active cross section, and the fourth root of the average fourth power of the lumen diameters expressed in μ (10^{-4} cm.). The average lumen diameters obtained from microscopical measurements also appear in the table for comparison with the calculated value. They were obtained by averaging arithmetically the radial and the tangential lumen diameters of several average radial rows of tracheids extending over an entire annual ring. About one hundred lumina were measured on each specimen. The values calculated from Poiseuille's law are consistently about 18 per cent lower than the directly

observed values. This is to be expected, since the calculated values are diameters of the uniform circular capillaries that would give the observed flow, whereas the actual cross sections are by no means circular; in the springwood they approach square and in the summerwood are rectangular with adjacent sides definitely unequal, to a varying extent.

Measurements were made using 95 per cent alcohol and benzene in place of water to see if a difference in surface wetting or surface viscosity might in any way affect the results. No appreciable effect appeared; the values thus obtained for the diameter of the lumen are very nearly the same as those obtained with water, showing that any differences in specific surface viscosity that may exist are of a magnitude insufficient to affect the results. The viscosity of the liquid itself seems to be alone effective.

EFFECT OF APPRECIABLE INCREASE IN THICKNESS

The effect of increasing the thickness of the transverse sections beyond that of a tracheid length was studied. In wood membranes of such thickness a number of tracheid lumina are connected in series through the small openings in the pit membranes, and the question of what capillary dimensions are effective in determining the rate of flow of liquids for such a system thus arises. It is answered by some experiments of Ewart,¹² who found that under a head of 80 cm. of water 4.0 to 4.2 cc. of water flowed per hour through a capillary tube having a radius of 0.0075 cm. and length of 8 cm. The theoretical flow for these conditions was 4.3 cc. per hour. The tube was then cut into 16 lengths of 0.5 cm. and each length was sealed into a tube 0.2 cm. in radius and 5 cm. long. All of these units were connected in series, thus giving a discontinuous capillary. Under the same head of water 3.8 to 3.9 cc. passed through the tubes per hour, showing substantially that only the fine capillaries are effective in determining the rate of flow, and that the rate is practically the same as for one capillary with a length equal to the sum of the lengths of the sections, increased impact turbulence accounting sufficiently for the small variation. When the liquid flows from tracheid to tracheid through the openings in the pit membranes, the situation is similar. The sizes of the openings in the pit membranes should be the determining factor, since the lumen capillaries, relatively speaking, are quite large. The effective length will no longer be the thickness of the section but rather the sum of the thickness of all the pit membranes that are traversed in series. Since the thickness of the pit membranes is minute, it cannot be determined with any great accuracy. An average value of the thickness of the middle lamella was taken as a fairly representative value of the membrane thickness, namely

¹² *Trans. Roy. Soc. (London)*, 198 B, 41 (1906).

0.5μ (0.5×10^{-4} cm.). It was assumed that the liquid flowed through approximately half the length of a tracheid in flowing from one tracheid to another. According to this the total membrane thickness X_m cm. traversed by the liquid passing through a transverse section of thickness l and having an average tracheid length t may be expressed approximately by the formula,

$$X_m = 0.5 \times 10^{-4} \left(\frac{l}{0.5t} - 1 \right) \quad [10]$$

For sections thicker than the maximum tracheid length, Equation 9 may be employed in calculating the effective size of the pores in the pit membranes by substituting r_m , the radius of the pores, for r_1 and X_m , the total pit membrane thickness, for l_1 thus:

$$r_m = \sqrt{\frac{\pi r_2^4 X_m P_2}{q_m l_2 P_1}} \quad [11]$$

If there are any open tracheids extending across the section, the expression becomes more complicated since flow then takes place both through the pit membranes and the few open tracheids in parallel. Combining the two kinds of wood capillary structure in parallel with the standard capillary in series Equation 8 becomes

$$\frac{\pi r_2^4 P_2}{l_2} = \frac{q_1 r_1^2 P_1}{l_1} + \frac{q_m r_m^2 P_1}{X_m} \quad [12]$$

where q_1 represents the total effective capillary cross section of the open tracheids and q_m the total effective capillary cross section of the pores in the pit membranes. The value of q secured from the electroendosmosis experiments is equal to the sum of q_1 and q_m . The measured value of q for sections thicker than the maximum tracheid length will be equal to q_m in the case of softwoods free from resin ducts. For the thinner sections q_1 can be obtained with sufficient accuracy by subtracting the minimum value of q (q_m) from the value of q for that section.

THE EFFECTIVE CAPILLARY DIMENSIONS OF THICK TRANSVERSE SECTIONS

Table IV gives the calculated diameter (fourth root of the average fourth power) of pit membrane pores in wood sections exceeding the maximum tracheid length in thickness, for two softwoods free from resin ducts. The variation in the values is not excessive even when 95 per cent alcohol and benzene are substituted for water, and the values show no regular variation with thickness of sections. These values conform well with those secured by other investigators for various membranes. Gueront⁷ obtained 14 to 20 $m\mu$ for the pore diameters of pig bladder, 7 to 17 $m\mu$ for goldbeater's skin, and 21 to 26 $m\mu$ for parchment paper. Ducleaux and Errera⁹ report 26 $m\mu$ for the pore diameter

TABLE IV.—Average Diameters of the Pores of the Pit Membranes.

Species	Thickness of Wood Section Cm.	X_m $\times 10^4$ Cm.	$\frac{r_2^4}{l_2}$ $\times 10^9$ Cm.	$\frac{P_2}{P_1}$	q (in per- centage of total active area of wood section)	Average Diameter of Pores of Pit Mem- branes $m\mu$
Western red cedar..	1.090	2.44	0.81	0.010	0.52	15
Do.....	1.067	2.38	1.99	.0022	Do.	11
Do.....	0.795	1.65	1.99	.0045	Do.	13
Do.....	0.610	1.15	0.81	.024	Do.	16
Do.....	0.587	1.08	1.99	.011	Do.	16
Do.....	*1.050	2.33	1.93	0.010	Do.	23
Do.....	*0.678	1.33	1.93	.018	Do.	23
Do.....	†0.988	2.17	1.93	0.005	Do.	16
Do.....	†0.647	1.25	1.93	.010	Do.	17
Alaska cedar	1.218	3.41	1.93	0.0017	0.34	14
Do.....	0.873	2.31	1.93	.0035	Do.	17
Do.....	0.582	1.38	1.93	.0090	Do.	21

* 95 per cent alcohol used instead of water.

† Benzene used instead of water.

of cellulose acetate membranes. Hitchcock¹⁰ obtained diameters ranging from 5 to 40 $m\mu$ for collodion membranes of different thicknesses. From this it is seen that the pit membranes have a capillary structure similar in pore dimension magnitudes to those of the natural animal membranes and of synthetic cellulose derivatives.

The results obtained over the entire range of thickness investigated with transverse sections are plotted in Figure 4 for two woods free from resin ducts. Up to a section thickness equal to the maximum tracheid length, a critical value, the decreasing effective capillary diameters represent the effective tracheid lumen dimensions. In the case of sections thicker than the maximum tracheid length, the diameters represent those of the pores in the pit membranes traversed. The sharp breaks in the curves occur at the respective maximum fiber lengths; the values are practically the same as those obtained in the previous electroendosmosis investigation (see Table I). The sharpness of each break is due to the fact that the total pit flow is practically negligible in comparison with that through only a few open tracheids. The more gradual transition in the upper part of each curve results from the fact that an increasing proportion of the open tracheids are cut across near one of their tapering ends as the thickness of the sections increases, thus giving an effective diameter which deviates more and more from the average diameter of a tracheid lumen.

OVERCOMING SURFACE TENSION IN CAPILLARIES

Another dynamic physical method, namely, that of overcoming the surface tension of a liquid in capillary systems, makes it possible to de-

termine the largest minimum capillary dimensions. Such figures furnish a check on the pore diameter sizes previously determined and give also

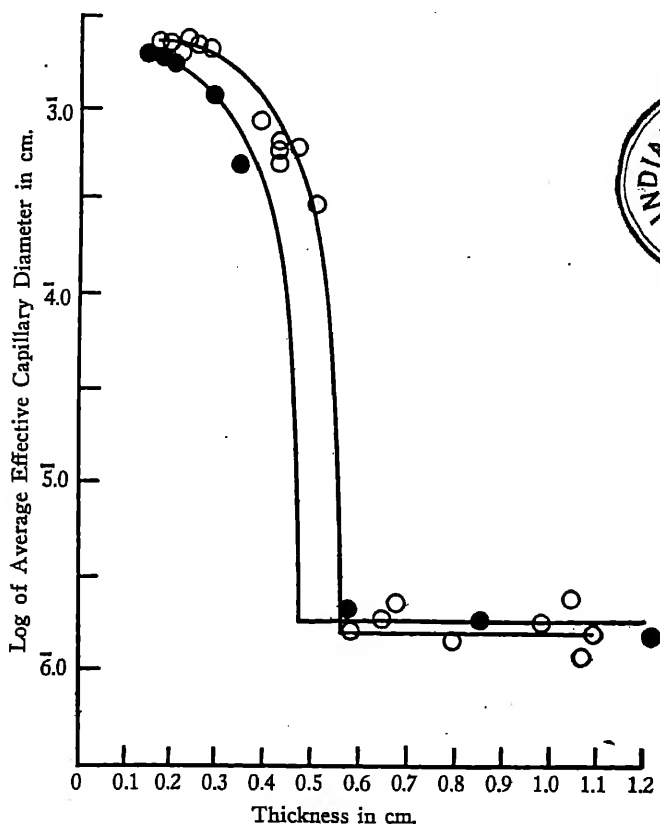


FIG. 4.—Change of Average Effective Capillary Diameter with Thickness of Transverse Wood Sections.

● Alaska Cedar, ○ Western Red Cedar.

a general idea as to the order of variation between average and largest minimum effective continuous capillary dimensions.

JURIN'S LAW

If the surface tension, σ , of a liquid that wets a capillary tube is known, the radius r of the capillary at the meniscus can be determined by measuring the height, h , of the capillary rise.

$$r = \frac{2\sigma}{h\delta g}$$

where d is the density of the liquid, and g is the acceleration of gravity. This method, however, cannot be used in the case of wood capillaries because their opacity prevents the measurement of h and also because the irregularity of their capillary systems makes it practically impossible to bring the liquid menisci to the desired points for measurement. It is possible, however, to measure the gas pressure required to overcome the capillarity. The applied pressure that will just cause the removal of the liquid from a single capillary and thus will barely allow the passage of gas is the pressure that will overcome the surface tension in the smallest part of the capillary bore. If several capillaries of different bores are combined in a bundle, the pressure that will just cause the removal of liquid, allowing the passage of gas, will correspond to that required to overcome the surface tension in the tube with the largest minimum bore, which is the same as the maximum effective bore because of the fact that the minimum bore of any irregular capillary tube determines its effectiveness. The water will be displaced first from the tube of least capillary resistance and the pressure required to do this is the only one that can be directly determined. Thus for the complex capillary systems in wood this method of determining capillary dimensions will always give the largest minimum bore for each of the systems of open capillaries subjected to the gas pressure. The pressure Q (per unit of area) is directly determinable from the equation,

$$Q = \frac{2\pi r \sigma}{\pi r^2} = \frac{2\sigma}{r} \quad [14]$$

For measurements made at 25° C. with water as the liquid to be displaced

$$r = \frac{2\sigma}{Q} = \frac{2 \times 72.1}{Q}$$

or

$$r \text{ (in } \mu\text{)} = \frac{1.442}{Q \text{ (in bars)}} = \frac{1.472}{Q \text{ (in kg. per cm.}^2\text{)}} = \frac{108.3}{Q \text{ (in cm. mercury)}} \quad [15]$$

PREVIOUS APPLICATIONS OF THE METHOD

The determination of pore diameters by means of Jurin's law has been studied by Bigelow and Bartell.¹³ Using glass capillaries with diameters ranging from 0.008 cm. to 0.114 cm., they found that the observed and the calculated diameters check quite well, and that the length of the capillaries has no effect. Further investigations by Bartell¹⁴ and by Bartell and Carpenter¹⁵ show the effect of the method of preparation of collodion membranes upon the pore diameters obtained by applying Jurin's law. Bartell and Osterhof¹⁶ have recently used this means of

¹³ *J. Am. Chem. Soc.*, 31, 1194 (1909).

¹⁴ *J. Phys. Chem.*, 16, 318 (1912).

¹⁵ *J. Phys. Chem.*, 27, 252 (1923).

¹⁶ *Z. phys. Chem.*, 130, 715 (1927); *Ind. Eng. Chem.*, 19, 1277 (1927).

determining pore diameters in membranes made up of compressed granular particles of carbon and silica. Their results check quite well with the pore diameters obtained by observing the permeability of the membranes to liquids under hydrostatic pressure and applying Poiseuille's law.

APPARATUS

The essential part of the apparatus appears in Figure 5. It consists of a "T" tube made of brass with a flange on one arm for clamping the wood sections securely to the tube. The cross section of the tube terminating in the flange is 0.8 sq. cm.; the applied pressure is operative over this area. The disk plate that holds the wood section in place by means of four screws has in it several small holes distributed over the area of the tube, to allow the free passage of air while still supporting the membrane enough to prevent complete rupture at high pressures. Pure gum rubber gaskets were clamped between the flange faces and the wood sections to furnish a practically air-tight seal. For thin transverse sections of wood, less than a tracheid length in thickness, the gas pressure was supplied from the laboratory air source and the pressure was measured on a mercury or a water manometer. For thicker sections of less permeability a high-pressure tank of oxygen with a readily controllable needle valve was used for the source of gas pressure and a calibrated pressure gauge with a range from 1 to 100 kilograms per sq. cm., which could be read to 0.5 kilogram per sq. cm., was used for determining the pressure.

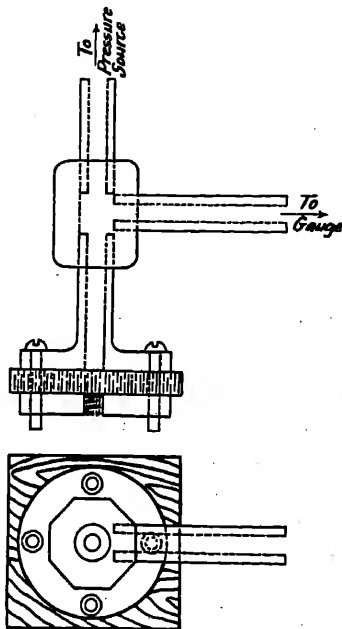


FIG. 5.—Apparatus for Overcoming the Surface Tension of Liquids in the Capillary Structure of Wood.

EXPERIMENTAL PROCEDURE

In making the measurements with water, a water-soaked section was clamped securely between the plates, and the apparatus was then dipped into a dish of water just far enough for the liquid barely to touch the

bottom of the wood section. The gas pressure was applied gradually, permitting accurate observation of the pressure at which gas bubbles emerged initially from under the lower plate. By using this technique, it was readily possible to distinguish actual passage of gas through the section from any slight gas leak between the upper plate and the wood.

The rate at which the pressure on the wood membrane is built up has some effect upon the pressure at which gas bubbles appear, an increased rate causing the pressure to reach a higher value before the bubbles appear. To prevent the building up of pressure faster than it could be dissipated, in working with the most resistant sections the needle valve was so operated that attaining the final pressure took several minutes. This lag was not so noticeable with the less resistant sections as with the others.

After making a determination the apparatus was disconnected from the pressure line and a drop of water was placed in the tube to assure the re-soaking of the upper part of the section. All of the pressure readings presented in this paper are averages of several values. For highly resistant sections giving pressure readings above 20 kg. per cm.² the values were usually checked to better than 6 per cent and for low-resistance sections to better than 2 per cent. In a few cases high-resistance sections gave a much smaller pressure reading on the second determination than on the first; all such values were rejected. In these cases careful examination of the section showed that rupture had taken place during the first measurement, which usually happened when the pressure was built up too rapidly. Such rupture should at times be expected since pressures of 50 kg. per cm.² are of the same order of magnitude as the shearing strength of some of the green softwoods.

MEASUREMENTS ON THIN TRANSVERSE SECTIONS

Measurements of the pressure required to overcome the surface tension of water in the open lumen capillaries of sections less than a tracheid length in thickness are given in Table 5, together with the corresponding calculated lumen diameters. Each of these diameters represents the largest minimum capillary bore of the bundle to which pressure was applied, *i.e.*, the maximum effective bore, for the approximately one hundred thousand tracheids making up the 0.8 sq. cm. cross section. The maximum lumen diameters obtained from the microscopical measurements made for the second part of this paper are also given. Such maximum values naturally are less than those obtained from the statistically larger number of lumina that probably are effective in the actual pressure measurements and that may include abnormal structure not considered in the microscopical measurements; the table shows definitely that they are less.

TABLE V.—*Maximum Effective Lumen Diameters Determined by the Application of Jurin's Law and by Direct Measurement.*

Species	Thickness of Wood Section	Pressure Required to Overcome the Surface Tension	Maximum Effective Diameter of Lumen	
			Jurin's Law	Direct Measurement
	Cm.	Cm. of Hg	μ	μ
Western red cedar	0.182	5.3	40.9	38.0
Do.....	.218	5.7	38.1	
Do.....	.269	6.0	36.1	
Do.....	.355	6.5	33.4	
Do.....	.420	9.6	22.6	
Alaska cedar	0.147	6.1	35.5	32.2
Do.....	.185	6.5	33.4	
Do.....	.231	7.8	27.8	
Do.....	.270	9.3	23.3	
Do.....	.318	12.6	17.2	
Do.....	.356	17.3	12.5	
Sitka spruce	0.172	4.5	48.3	40.8
Do.....	.198	5.0	43.3	
Do.....	.262	5.2	41.7	
Do.....	.317	5.8	37.4	
Western yellow pine...	0.178	4.1	53.0	43.8
Do.....	.241	5.1	42.5	
Do.....	.267	4.8	45.2	
Douglas fir (Rocky Mountain type)	0.183	5.7	38.1	35.0
Do.....	.198	5.8	37.4	
Do.....	.274	7.4	29.3	

Measurements were made using benzene-soaked sections as well as water-soaked ones. When an approximate correction for the difference in swelling caused by the two liquids was made, the pressure required to remove each of the liquids was found to be approximately proportional to its surface tension. This indicated that the angle of wetting of wood by benzene is either quite small or is zero, provided that no film of water sufficiently thick to prevent the formation of a benzene-wood interface remained on the benzene-soaked sections, which were dried to constant weight at 105° C.

MEASUREMENTS ON THICK TRANSVERSE SECTIONS

Measurements were made on transverse sections thicker than the maximum tracheid length. In this case the pressure required to just displace the water from the irregular systems of tracheid lumina and pit membrane pores is that required to overcome the surface tension in the smallest part of the bore of the most effective combination of pores in series. Table VI gives the results of these measurements and the calculated maximum effective pore diameters of the pit membranes; the

TABLE VI.—*Maximum Effective Diameters of the Pores of the Pit Membranes Determined by the Application of Jurin's Law.*

Species	Thickness of Wood Section	Pressure Required to Overcome the Surface Tension	Maximum Effective Diameter of the Pores of Pit Membranes
	Cm.	Kg per cm. ²	mμ
Western red cedar.....	1.500	24	120
Do.....	1.067	28	103
Do.....	0.795	21	137
Do.....	.587	16	181
Alaska cedar	1.218	40	72
Do.....	0.873	43	67
Do.....	.582	37	78
Do.....	.450	15	160
Sitka spruce	1.103	30	96
Do.....	0.828	18	160
Do.....	.574	21	137
Do.....	.538	18	160
Western yellow pine.....	1.370	18	160
Do.....	0.835	16	180
Douglas fir (Rocky Mountain type)	1.310	43	67
Do.....	.735	28	103
Do.....	.520	24	120

values are four to eight times as great as the corresponding average values obtained in the hydrostatic flow study. Such differences in order of magnitude should be expected, since the pores are most probably not all of the same size. These values thus serve as a check upon the validity of the order of the average pit membrane pore diameters obtained by the hydrostatic-flow method.

Figure 6 gives the logarithms of the maximum effective (largest minimum) capillary diameters expressed in centimeters for a large range of thicknesses of transverse sections of two softwoods free from resin ducts. The curves are almost identical in shape with the similar curves for average effective diameters (see Fig. 4). A break occurs at the maximum tracheid length, since the change in effective pore diameters for thicker sections is slight, whereas the increase in capillary diameters for decreasing thicknesses is very rapid below the maximum tracheid length because of the inclusion of open tracheids in the system.

Determining the thickness at which the break in the curves of Figure 6 occurs furnishes a third independent method of obtaining the maximum tracheid length. The values are given in Table I, together with the data obtained by actual measurement as well as by the other dynamic physical methods.

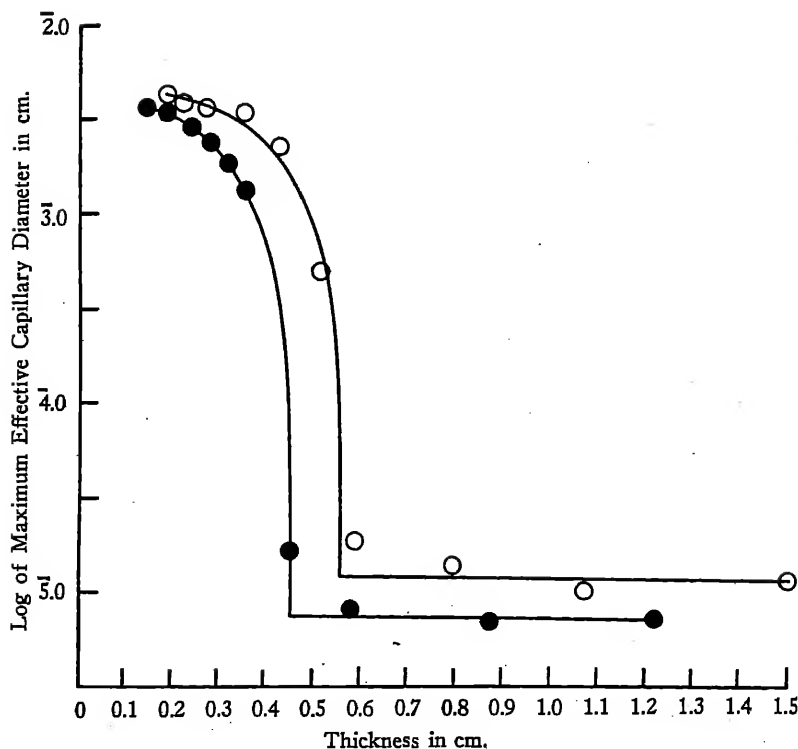


FIG. 6.—Change of Maximum Effective Capillary Diameter with Thickness of Transverse Wood Sections.

● Alaska Cedar, ○ Western Red Cedar.

PERMEABILITY OF WOOD TO COLLOIDAL SOLUTIONS

The permeability of wood to colloidal solutions containing particles of approximately known size was tested as a check upon the calculated capillary dimensions. A 0.02 gram per liter mercury sol prepared by the Bredig electrical condensation method with potassium citrate (0.0025 normal) as a stabilizing agent was used.¹⁷ The average particle size, 8 $m\mu$, for this sol, the particles of which were quite uniform in size, was obtained by the method of fluctuations developed by Smoluchowski.¹⁸ Nephelometric analysis showed no change in the concentration of the sol when wood sawdust was added. The fact that the sol is not perceptibly coagulated by wood should be expected, since both are electro-

¹⁷ This sol was obtained from Mr. F. W. Laird of the Chemistry Department of the University of Wisconsin. He also determined the particle size.

¹⁸ Svedberg, *The Colloid Chemistry*, New York, The Chemical Catalog Co., Inc., 1924, pp. 118-127.

negative with respect to water. A ferric oxide sol, which is electro-positive with respect to water, was coagulated by wood and hence could not be used.

The passage of the mercury sol through the thick transverse sections of western red cedar, Alaska cedar, sitka spruce, and Douglas fir was tested, using the apparatus of Figure 5. Ten cubic centimeters of the sol was placed in the tube above the membrane and a pressure of one-half atmosphere was applied. In one case 9.8 cc. of the sol passed through a section in 18 hours, with a reduction in concentration to about one-tenth its original value. This, as well as other experiments that gave similar results, shows that the sections, although somewhat permeable, also act as partial ultrafilters. Owing to the electrical repulsion of the mercury particles and the wood surface, which are both negatively charged, it is necessary for the capillary openings to be somewhat larger than the colloidal particles in order for the latter to pass through freely. For such small dimensions the openings may in fact have to be several times the diameter of the particles. The average pore diameters previously obtained ranged from 12 to 20 $m\mu$. Openings of such size may readily hold back charged particles 8 $m\mu$ in diameter. The maximum pore diameters reported in this paper, which are four to eight times as large as the average values, can, however, hardly be expected to be impervious to the mercury sol. The fact that partial filtration is obtained, therefore, furnishes a check upon the order of magnitude of the pore diameters obtained by the two preceding methods.

A further check upon the partial filtration effect was obtained by placing some of the sol in the tube above the membrane (see Fig. 5) and applying a pressure of the order of that required to blow gas through the wet sections. An Alaska cedar water-soaked section that had previously required 41 kg. per $cm.^2$ to displace the water and allow the passage of gas required 42 kg. per $cm.^2$ for gas bubbles to appear after placing a little of the sol in the tube. The resulting opacity of the water into which the section dipped indicated the passage of the sol. The experiment was repeated several times; each time a greater pressure was required for the gas to pass, and the amount of mercury sol passing through decreased. Finally, no gas would pass through the section below 52 kg. per $cm.^2$, at which pressure the section broke. Practically no mercury particles passed through the section on the last application of pressure. Similar results were obtained with other sections. Substituting India ink for the mercury sol also gave similar results except perhaps for the fact that the sections were slightly more permeable to it than to the mercury sol. These experiments thus show that part of the continuous longitudinal capillary structure of softwoods is sufficiently coarse to allow the passage of colloidal mercury solutions and of India ink, but nevertheless part of the structure is sufficiently fine to serve as a

partial ultrafilter. Continued pressure filtration, however, will eventually cause the clogging of even the larger capillaries, so that the sections finally become practically impervious. The fact that some India ink can be forced through the continuous longitudinal structure is a confirmation of the results obtained by Bailey.³

CONCLUSIONS

The investigation reported here, in which four different dynamic physical methods of studying the structure of wood have been developed and applied, shows definitely that considerable information on the structure of wood can be obtained by means other than microscopical. These methods, moreover, can be extended to the study of structure below the range of microscopical visibility. The justification for the use of these physical methods, each of which is known to be valid for coarser structure, has been established sufficiently by the fact that the independent methods gave consistent results. For example, the *average* effective continuous capillary diameters for longitudinal flow, obtained by combining the electroendosmosis data for the total capillary cross section with the pressure-permeability data and applying Poiseuille's law, are quite comparable with those for the *maximum* effective continuous capillary diameters obtained by overcoming the surface tension of water in the wood capillaries. The two methods are entirely independent and though each measures slightly different structure, nevertheless they give the type of variations that would be expected. The first depends upon the assumption that the passage of liquid from tracheid to tracheid in thick sections of softwood is primarily through an effective capillary length equal to that of the combined pit-membrane thicknesses traversed in series. The second involves no such assumption. The results thus tend to show also that the major part of the flow from tracheid to tracheid is through the pits. Microscopical examination has suggested this but has never proved it conclusively.

The physical methods developed, besides having the decided advantage of extending the study of capillary dimensions below those of microscopical visibility, have the further advantage of giving results more of a statistical nature than ordinary microscopical examination would give. For example, the measurement of the maximum, minimum, and average tracheid lengths by the electroendosmotic means is automatically obtained in a statistical form for about one million tracheids. The other two dynamic methods of obtaining the maximum tracheid length, namely, by hydrostatic flow studies and by overcoming the surface tension in the capillaries, likewise give values for a similarly large number of tracheids. Further, the average effective lumen diameters and the maximum effective lumen diameters as obtained by these physical meth-

ods are likewise far more statistical in nature than values determined by ordinary microscopical methods.

The major part of this investigation has been devoted to the development of methods. Further work in progress is obtaining data for different species, comparing heartwood with sapwood, and green wood with re-soaked seasoned wood. These data when more complete will undoubtedly help in characterizing the capillary structure of wood and in explaining the complex pit mechanism.

SUMMARY

Electroendosmotic Flow. The total effective capillary cross sections of several different softwood sections cut in each of the three structural directions were determined by means of electroendosmotic-flow studies. From the change in rate of electroendosmotic flow with changes in thickness of transverse sections, the maximum, minimum, and average tracheid lengths were determined, as well as the effective continuous pit-communication cross section.

Hydrostatic Flow. Combining the electroendosmotic-flow data with those obtained from hydrostatic-flow studies, the average effective lumen diameters and the average effective pit-membrane pore diameters were determined. The former agree quite well with values obtained by microscopical measurement. The latter are of the same order of magnitude as the pores in other natural and artificial membranes. Maximum tracheid lengths obtained from the hydrostatic studies agree well with the values obtained by the electroendosmotic method.

Overcoming Surface Tension of Water in the Capillary Structure. With the surface-tension method of investigation the maximum effective lumen diameters and maximum effective pit-membrane pore diameters were determined. The former agree well with values obtained microscopically. The latter are four to eight times as large as the average effective values obtained by the other methods. This physical method, which furnishes a third independent dynamic means of determining the maximum tracheid length, gives values that agree well with the other values.

Permeability to Colloidal Solutions. Though the wood sections studied are somewhat permeable to colloidal mercury and to India ink sols, they also act as partial ultrafilters. This fact should be expected, since the mercury particles, although about a tenth as large as the largest pit-membrane pores, are of the same order of size as the average effective pit-membrane pore diameters.

*United States Forest Products Laboratory,
Madison, Wisconsin.*

FRACTIONATION OF DIPHTHERIA ANTITOXIC PLASMAS

By P. J. MOLONEY AND EDITH M. TAYLOR

The results which are reported in this paper have to do with the purification of diphtheria antitoxin by fractionation with alcohol and with tannic acid; and also with the possibility of modifying antitoxic protein—derived from horse plasma—so that serum reactions following its administration to humans might be less apt to occur.

FRACTIONATION WITH ALCOHOL

From preliminary experiments it was found that by the use of ethyl alcohol under suitable conditions a considerable portion of non-antitoxic protein could be precipitated directly from antitoxic plasma.¹ The results in Table I have to do with precipitations of this kind carried out under various conditions. Citrated plasma (700 units per cc.) diluted 1:5.5 with water was used in these experiments; the pH's were varied by the addition of sodium acetate and acetic acid; the final solutions were 0.14 molal acetate.

TABLE I. *Yield and Purity of Antitoxin in Supernatant from Plasmas Precipitated by Different Amounts of Alcohol at 40°C. at Various pH's.**

pH	Alcohol 14.4 Per Cent		Alcohol 17.3 Per Cent		Alcohol 22.4 Per Cent		Alcohol 26.8 Per Cent	
	Per Cent	Units	Per Cent	Units	Per Cent	Units	Per Cent	Units
	Yield	per Gram	Yield	per Gram	Yield	per Gram	Yield	per Gram
4.77	83	13000	54	11600	38	6
5.27	100	15000	80	15000	60	16000	<40
5.68	100	12700	100	14000	70	13850	<40
6.01	100	12200	100	14000	70	13500	40	13600

* Original plasma 11500 units per gram.

It will be noted that on the basis of yield and purity the best condition as shown in the above Table for this first fractionation are pH 5.3 and 14 per cent alcohol. When precipitation on the same solution is carried out at room temperature, the optimum conditions are pH 5.3 and 27 per cent alcohol.

With solutions purified by the method indicated in Table I experiments were carried out on the stability of antitoxin with respect to

¹ The plasmas used contained 100 cc. of 5 per cent sodium citrate solution per liter of blood. In no case was a preservative added.

hydrogen-ion concentration and to alcohol concentration. These data are given in Table II.

The solutions used in these experiments were prepared in the following way: Diluted antitoxic plasma pH 5.0 and alcohol concentration 17 per cent was heated for 30 minutes at 40° C. The precipitate which formed was removed by centrifuging and discarded. The antitoxin contained in the supernatant was freed, in great part, from salts by precipitation at 8° C. with alcohol to a concentration of 50 per cent. The precipitate was dissolved in water to a protein concentration of 0.7 per cent. The hydrogen-ion concentration of the acid solutions were obtained by the addition of hydrochloric acid and those of the alkaline solutions by the addition of sodium hydroxide.

TABLE II. *Stability of Antitoxin Under Various Conditions.*

pH	Room Temperature 16 Hours						40° C.	55° C.
	No	4	8	14.3	19.8	24.6	45 Min.	45 Min.
	Alcohol	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	No	No
	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Per Cent	Alcohol	Alcohol
1.54	<40	<40	<40	<40	<40	<40
1.91	<40	<40	<40	<40	<40	<40
3.13	70	50	40	<40	70	...
3.40	90	80	65	45	75	...
3.64	95	80	65	45	75	...
4.16	100	100	100	100	100	75
4.83	100	100	100	100	100	90
10.96	100	...	100	100	100	100
11.95	40
12.45	<40
12.81	<40

It will be noted that in the acid solutions, at pH 4.16, there was no destruction of antitoxin under the conditions given, except in the solution which was heated to 55° C. for 45 minutes. At pH 3.40 there was more destruction of antitoxin in the solutions containing higher concentrations of alcohol than in those of lower alcohol content. In the alkaline solution, pH 11.95 and greater, there was destruction of antitoxin after standing for 16 hours at room temperature. In the solutions pH 10.96 there was no loss of antitoxin even in the solutions which contained 24 per cent alcohol.

It was found that on neutralization antitoxin which had first been treated with acid was more insoluble than that which had been treated with alkali.

In order to investigate this phenomenon more fully, the following experiments were carried out. A relatively salt-free solution of antitoxin, containing 0.7 per cent protein, was prepared in the same way as that for the experiments in Table II. One part of this solution was ad-

justed with hydrochloric acid to pH 3.5 and alcohol added to 14 per cent concentration; a second part of the solution was adjusted to pH 10.5; the remainder of the solution was left unaltered. All three solutions were allowed to stand at room temperature for 16 hours. The solution pH 3.5 after standing 16 hours at room temperature was divided into 2 parts. One part of this was adjusted to pH 10.2 and allowed to stand 16 hours at room temperature. The three solutions which had been treated with acid and alkali were adjusted to approximately pH 6.0 and the protein contained in these was precipitated in the cold by the addition of alcohol. The three precipitates were dissolved in water to original volumes. With the four solutions of antitoxin, *i.e.*, the original solution to which no alkali or acid had been added, and the three solutions which had been adjusted to pH 3.5, 10.5 and 3.5-10.0 respectively, the following experiment was carried out:

Aliquots of each solution were adjusted to a number of different pH's by diluting 4:5 with phosphate-citrate buffers (McIlvaine); alcohol was added in each case to a concentration of 8 per cent. All the solutions were chilled to 8° C. and the precipitates removed by centrifuging in the cold.

With each solution antitoxin was precipitated over a broad zone of pH. The zones of precipitation of antitoxin for the different solutions are shown in Table III.

TABLE III. *Zones of Maximum Precipitation of Antitoxin.*

Solution	pH Range	Antitoxin Precipitated
Original solution	4.6 to 5.6	6 per cent
Acid solution	5.2 to 6.7	8 per cent
Alkaline solution	5.1 to 5.7	Trace
Acid-alkaline solution	5.1 to 5.7	Trace

It will be noted that the zone of maximum precipitation for the solution which had first been adjusted to pH 3.5 (acid solution) was distinctly more alkaline than the zone of maximum precipitation for the original solution. When this same solution (acid solution), adjusted to pH 10.2, had stood for 16 hours at room temperature (acid-alkaline solution) there was a marked increase in the solubility of the antitoxin.

FRACTIONATION WITH TANNIC ACID

Kruyt² in a paper on "Unity in the Theory of Colloids" at the 1927 colloid symposium presented interesting data on the effect of tannin on the stability of emulsoids. In view of the emulsoid properties of

² Colloid Symposium Monograph, Vol. 5, Chemical Catalog Co., New York, 1927, p. 1.

antitoxic protein as indicated by the type of precipitation induced by alcohol, it was thought worth while to investigate the action of tannin on solutions of antitoxin. When this work was undertaken, tannin was not immediately available and instead tannic acid was tried. This gave results of interest and was used throughout. The first point investigated was the effect of tannic acid on the solubility of antitoxin. The results given in Table IV are self-explanatory. The solution of antitoxin used was the same as that used for the experiments recorded in Table II. It contained 0.7 per cent protein and was relatively salt-free. Tannic acid was added to 0.14 per cent concentration.

TABLE IV. *Precipitation of Antitoxin with Tannic Acid at Various pH's.*

pH	17 Per Cent Alcohol Per Cent Antitoxin in Precipitate	No Alcohol Per Cent Antitoxin in Precipitate
4.44.....	80	75
4.86.....	100	98
5.25.....	95	95.5
5.44.....	65	45

It will be noted that in the series recorded, the solubility of the antitoxin in the presence of tannic acid is at a minimum at pH 4.8 to pH 5.2 in either alcoholic or aqueous solution, and that the pH zone of insolubility of the antitoxin is broader in alcoholic solution.

Further, the solubility of antitoxin in the presence of tannic acid is a function of both temperature and salt concentration, *i.e.*, more tannic acid is required to precipitate antitoxin from solution at 37° C. than at room temperature and more at room temperature than at 8° C.; and within certain limits, less tannic is required to precipitate antitoxin from a salt-free solution than from a solution containing dissolved salts.

The following is an example of one method of purifying antitoxin by the use of tannic acid. Thirty cc. of a plasma which contained 11,500 units of antitoxin per gram solids was diluted with 142.5 cc. distilled water. The pH was adjusted to pH 5.3 by the addition of sodium acetate and acetic acid to 0.14 molal acetate. Alcohol was added to give a concentration of 17 per cent. The solution after heating to 40° C. for 30 minutes was centrifuged and the precipitate discarded. The supernatant was adjusted to pH 5.0, chilled to 8° C., and tannic acid added to a concentration of 0.14 per cent. The precipitate which formed was centrifuged in the cold. The supernatant was discarded and the precipitate stirred for 16 hours at 37° C. with 210 cc. phosphate-citrate buffer (McIlvaine) pH 5.8 diluted 1:4 with distilled water. The undissolved portion was removed by centrifuging. The supernatant contained 60 per cent of the original antitoxin; the purity of this was 24,000 units of antitoxin per gram of solids. A small amount of tannic acid was present in this solution.

The degree of purity of the antitoxins which we have obtained by the use of alcohol or tannic acid has been in the order of that obtained by fractionation with ammonium sulfate or sodium sulfate. This degree of purity is markedly less than that reported by Ramon³ and Locke and Main,⁴ using methods based on specific precipitation of diphtheria antitoxin by diphtheria toxin.

In view of the precipitating qualities of tannic acid on antitoxin it is of interest to present results on the precipitation of antitoxin with the acid dye Bielrich scarlet. The results are shown in the graph.

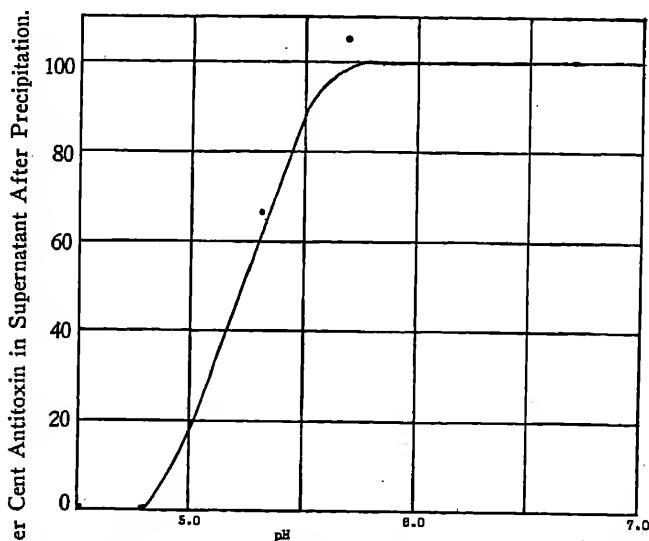


FIG. 1.—Precipitation of Diphtheria Antitoxin with Bielrich Scarlet.

These results in conjunction with the data on the minimum solubility of antitoxin in alcoholic solutions are in accord with the assumption that the isoelectric point of diphtheria antitoxic protein in the solution used is in the pH range 4.6–5.6. Maver and Falk⁵ have determined by cataphoresis experiments that at pH 6.0 diphtheria antitoxin is negatively charged and at pH 4.6 it is positively charged.

INTRADERMAL REACTIONS TO SOLUTIONS OF ANTITOXIN

At least two types of disturbance may follow an injection of antitoxin. One type, which fortunately is rare, is in the nature of a sudden

³ *Compt. rend. soc. biol.*, 88, 167 (1923).

⁴ *J. Infectious Diseases*, 39, 126 (1926).

⁵ *J. Immunitäts*, 14, 219 (1927).

collapse which comes on shortly after the administration of the antitoxin. In very rare instances death follows. The other type of disturbance usually does not show itself for 8 to 10 days after the antitoxin has been given. The most constant symptom is an itching skin rash, usually accompanied by a rise in temperature and joint pains. To this group of symptoms the name serum sickness is given.

From the standpoint of purity there was no reason to believe that any of the products which we have prepared would cause less serum reactions than the antitoxins which are used clinically, but in view of the marked change in the pH of minimum solubility of one of the products (Table III) it seemed possible that such an altered antitoxin might be less apt to cause serum reactions. While the results given below have to do with a type of serum reaction they may not bear directly on the problem of serum reactions which occur in the treatment of diphtheria.

Three individuals who gave marked reactions to an intradermal injection of diluted commercial antitoxin—purified by the ammonium sulfate method—were tested intradermally with dilutions of the solutions used for the experiments on minimum solubility in alcoholic solution (Table III). All the solutions used including the commercial antitoxin were diluted to contain 0.15 per cent protein. With two of these individuals there was markedly less reaction to the four solutions of antitoxin which had been purified by the alcohol method than to the dilution of the commercial antitoxin. Of the four antitoxins purified by the use of alcohol, the one which had not been treated with acid or alkali gave as little reaction as the antitoxins which had been so treated. With the third individual the reactions to all five intradermal injections were the same.

SUMMARY

The paper gives data having to do with the fractionation of diphtheria antitoxin with alcohol and with tannic acid; and with the stability of antitoxin with respect to pH and alcohol.

Solutions of antitoxin prepared by the use of alcohol gave, with certain serum-sensitive individuals, less reaction after intradermal injection than solutions of antitoxin prepared by the use of ammonium sulfate.

*Connaught Laboratories,
University of Toronto,
Toronto, Ontario, Canada.*

CATAPHORESIS OF BLOOD CELLS AND INERT PARTICLES IN SOLS AND GELS AND ITS BIOLOGICAL SIGNIFICANCE

BY HAROLD A. ABRAMSON

Although more than a century has elapsed since Reuss¹ first observed the migration of clay particles in an electric field, it is only in recent years through the experiments of Ellis² and the theory of von Smoluchowski³ that fairly good absolute values of the cataphoretic velocity of biological microscopic units, such as bacteria and white blood cells, have been obtainable. It is indeed fitting that the development of methods for measuring the cataphoretic velocity of living cells occurs when our knowledge of the behavior of electrolytes and non-electrolytes at phase interfaces is correspondingly progressing.

One of the most important microscopically visible processes occurring throughout the higher animal kingdoms is the acute inflammatory process. And one of the most striking parts of this process is the migration of the polymorphonuclear leucocyte through the gel-like structure of the blood capillary to the point of injury in or near the adjacent fibrous tissue. It is obvious that electromotive forces passing between injured and uninjured tissues could play an important, perhaps a dominant role, in bringing a charged leucocyte to the point of injury. In general, the problem concerned with the solution of such a proposed mechanism consists in studying the cataphoretic migration of microscopically visible particles through sols and gels of the types encountered in the



FIG. 1.—Diagrammatic Representation of Blood Cells. 1=polymorphonuclear leucocytes; 2=red cells and their aggregates; 3=small lymphocytes. The cataphoretic velocity of these types of cells is given in Table I.

¹ Reuss, *Mem. Soc. Imp. Naturalistes Moscou*, 2, 327 (1809). Quoted by Michaelis, L., in Alexander, "Colloid Chemistry," New York, The Chemical Catalog Co., Inc., 1926, p. 471.

² Ellis, *Z. physik. Chem.*, 78, 321 (1912). See also Powis, *ibid.*, 89, 91 (1915).

³ Von Smoluchowski in Graetz, "Handbuch der Elektrizität und des Magnetismus," Vol. II, 1921, p. 366.

tissues.^{4, 5, 6} The observations of Arrhenius⁷ and of McBain,⁸ Laing⁹ and co-workers show that for ions and colloidal micellae in certain sols and gels, the mobility is unchanged during the sol-gel transformation. These studies could help us predict but do not designate how microscopically visible particles from $1\ \mu$ to $30\ \mu$ in size would behave under similar circumstances. I have previously shown,⁸ making certain assumptions, that the order of magnitude of these electromotive forces arising in injured tissues should be sufficient to produce the movement of a white cell to a point of injury by cataphoresis.⁴ Since that time I have had the privilege of continuing these studies in the laboratories of Professor Freundlich at Dahlem. The researches to be reported here in résumé are those which I have done with Professor Freundlich.

THE CATAPHORESIS OF SINGLE CELLS IN HORSE SERUM⁵⁻¹⁰

In the following experiments a modification of the cemented cell described by Northrop^{10, 11} was employed. Northrop's method has facilitated cataphoretic measurements of microscopic particles a great deal.

The white blood cells of the horse are conveniently obtained and studied in serum (and oxalated plasma). Serum contains about 7 per cent of proteins in addition to the electrolytes which maintain it well buffered at about pH 7.4. One may distinguish easily amongst the white cells the two types that concern us at present. These are: (1) the amoeboid, irregularly shaped polymorphonuclear leucocyte (diameter approximately $10\ \mu$); and (2) the round single nucleated small lymphocyte (diameter about $8\ \mu$). The red cells are about $6\ \mu$ in diameter (Fig. 1). Following are the mean cataphoretic velocities observed for these types of cells under the above conditions. Keeping the cells on ice (in serum) up to three days did not change these values. (The cataphoretic velocities in such suspensions are proportional to the drop in potential (1-30 volts per cm.) Table I.

Red cell	1.00 μ per second per volt per cm.
Polymorphonuclear leucocyte	0.51 μ per second per volt per cm.
Small lymphocyte	0.60 μ per second per volt per cm.

Single values for the most irregularly shaped leucocytes fell within the experimental error, thus demonstrating an independent relationship

⁴ Abramson, *J. Exp. Med.*, 41, 445 (1925).

⁵ Abramson, *J. Exp. Med.*, 46, 987 (1927).

⁶ Abramson, *J. Gen. Physiol.* In press.

⁷ Arrhenius, *Öfvers. Stockholm Akad.*, 6, 121 (1887).

⁸ McBain, in Bogue, "The Theory and Application of Colloidal Behavior," New York, McGraw-Hill Book Co., 1924, p. 410 et seq.

⁹ Laing, *J. Phys. Chem.*, 28, 673 (1924).

¹⁰ Freundlich and Abramson, *Z. physik. Chem.*, 128, 25 (1927).

¹¹ Northrop, *J. Gen. Physiol.*, 4, 629 (1922).

TABLE I. Mean Cataphoretic Velocity, V , of the Cells of the Blood (Horse).

Type of Particle	Medium	V μ /Sec. Volt/Cm.	ξ -Potential Millivolts	Remarks
Red cell	Serum	1.01	24.5	Although just outside the experimental error, the differences noted for lymphocytes and leucocyte are measurable.
Polymorphonuclear leucocyte	Serum	.51	12.5	
Lymphocyte	Serum	.60	14.5	
Polymorphonuclear leucocyte	Oxalated plasma	.49	12.0	The differences noted for polymorphonuclear leucocytes and platelets are probably due to experimental error.
Platelet	Oxalated plasma	.45	11.0	
Red cell	Oxalated plasma	.98	24.0	

These values are perhaps slightly higher than those which could be obtained under more suitable experimental conditions.

ξ has been calculated as $V \times 24.5$ ($20^\circ\text{C}.$).

(No correction for the differences in viscosity between plasma and serum has been made. The η values for serum and plasma have been taken as 0.0185. D for serum is given as 85 by Fürth.)

Quartz particles in serum have the same cataphoretic velocity as polymorphonuclear leucocytes.

between shape and cataphoretic velocity. It is probable that these values are slightly higher than absolute values obtainable under better experimental conditions. Their comparative values however are most significant. They fit in with our previous knowledge of the surfaces of the red cell and leucocyte. They differ somewhat from previous investigations in that fairly accurately measurable differences in surface constitution of these cells have been directly obtained. In order to investigate the nature of these differences the migration of quartz particles in serum was studied simultaneously with that of the cells. It had been observed by Davis¹² that glass adsorbs serum protein on both sides of the isoelectric points of the proteins present. A similar observation was independently made. Polymorphonuclear leucocytes and quartz particles have the same cataphoretic velocity. The fact that leucocytes migrate with the same speed as quartz particles covered with serum protein is most suggestive. It indicates that while the surface of leucocytes consists of the protein of the serum, the red cell preserves its surface integrity. The recent experiments of Dr. and Mrs. Mudd¹³ show the hydrophobic nature of this surface strikingly. The slight difference between leucocyte and lymphocyte leads one to believe that the lymphocyte still retains some of its own surface properties. Just what proteins or

¹² Davis, *Proc. Physiol. Soc.*, Dec. 16, 1922, in the *J. Physiol.*, 58, 1923.

¹³ Mudd and Mudd, *J. Exp. Med.*, 43, 127 (1926); *Bioch. Z.*, 186, 378 (1927).

other substances have been adsorbed must be determined by future experiments with the fractions of the serum proteins on the cells themselves. In this connection it is interesting to note that blood platelets which play an important role in the coagulation of blood migrate also with the speed of leucocytes. This surface similarity may indicate a common ancestral cell.

THE INFLUENCE OF THE SHAPE OF THE PARTICLE ON CATAPHORESIS

According to the theory of Helmholtz¹⁴ as later modified by Pellat¹⁵ and von Smoluchowski,³ V , the cataphoretic velocity of a microscopic particle is

$$V = \frac{1}{4\pi} \cdot \frac{HD\zeta}{\eta} \quad [1]$$

where H = drop in potential, D = dielectric constant of the medium, ζ = electrokinetic potential, and η = the viscosity of the medium (units c.g.s. electrostatic). In this expression the cataphoretic velocity is independent not only of the size but also of the *shape* of the particle.

Debye and Hückel¹⁶ and Hückel¹⁷ have recently stated that von Smoluchowski was in error and that on theoretical grounds the factor $1/4\pi$ was valid for the case of a cylinder and $1/6\pi$ for the sphere. It was of fundamental importance to determine if the contention of Debye and Hückel, namely, that cataphoretic velocity is dependent upon the shape of the particle, was correct. For example in the case of the differently shaped leucocytes, if differences in velocity were dependent upon shape, it would be practically impossible to study them by this method, nor would the study of quartz particles which are irregular in shape be simple.

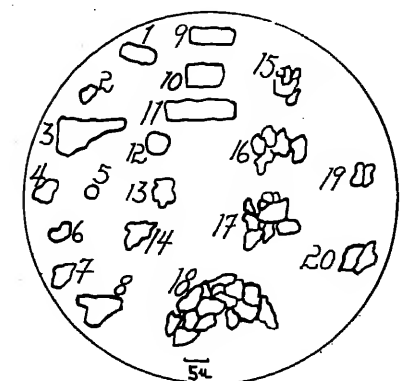


Fig. 2.—Quartz particles of different shapes, such as these, when suspended in a sugar solution, migrate with the same cataphoretic velocity (within the limits of experimental error). The values are given in Table II.

It has been noted that polymorphonuclear leucocytes having diverse shapes migrated independently of their shapes. This was the first contradiction to the theory of Debye

¹⁴ Helmholtz, *Wied. Ann.*, 7, 337 (1879).

¹⁵ Pellat, quoted by Perrin, *J. Chim. Phys.*, 2, 601 (1904).

¹⁶ Debye and Hückel, *Physik. Z.*, 25, 49 (1924).

¹⁷ Hückel, *Physik. Z.*, 25, 205 (1924).

and Hückel. A still more striking one is that demonstrated by the behavior of single red cells and their aggregates which have cylindroid (*rouleaux*) and frequently spheroid or irregular forms. Here again the velocity was independent of the shape of the particles whose sizes also varied considerably. These systems are biological. It was necessary therefore to determine whether these observations were confirmed in experiments performed under more ideal conditions. Quartz particles of the most different forms suspended in water and in sugar solutions (whose higher density permitted larger particles to be definitely outlined) migrated with practically identical velocities¹⁸ (Fig. 2). As the speed of the fluid within the cataphoresis cell varies appreciably at different levels, it is important that particles observed be studied at the same level, preferably in the center where the change is least.

TABLE II. *Relative Speed of Larger Quartz Particles and Aggregates in Sugar Solutions.*

Particle	Relative Speed for 50 μ Distance in Seconds
1.....	10.8
2.....	10.9
3.....	10.4
4.....	10.5
5.....	11.0
6.....	11.3
7.....	11.2
8.....	12.0
9.....	11.2
10.....	11.4
11.....	11.0
12.....	12.0
13.....	11.2
14.....	11.4
15.....	These aggregates migrate (within the limits of experimental error) with the same speed as single particles sus- pended at the same level.
16.....	
17.....	
18.....	
19.....	
20.....	

The numbers refer to the particles similarly numbered in Figure 2.

The migration of all the types of particles takes place under normal conditions without orientation. If, however, the drop in potential per cm. in the cataphoresis cell is increased, with increased waterflow, as well as increased velocity, orientation does take place. This absence of orientation is particularly strikingly observed in soft gelatin gels (to be described later) where large elongated particles maintain the same direction during hours of study. The particles of the sizes studied (1μ -

¹⁸ Freundlich and Abramson, *Z. physik. Chem.*, 133, 51 (1928).

30 μ) are not oriented by the electrical field. Any orientation occurring can be ascribed to secondary causes.

It may be recalled that the particles of vanadium pentoxide sols orient themselves in an electric field.²⁰

It seems possible that the large size of the particles here investigated may have caused a statistically symmetrical response to the applied field. Just how diminishing the size of the quartz particles would influence their orientation in the field is unknown. Similar experiments with the ultramicroscope may decide this question.

THE RELATIONSHIP BETWEEN ENDOSMOTIC AND CATAPHORETIC VELOCITY

It follows from the two equations submitted by Debye and Hückel, and Hückel, that if one were to construct a system where the velocity of particle cataphoresis and of endosmosis along a wall having the same surface as the particle,

$$3V_p = 2V_w \quad [2]$$

where V_p = cataphoretic velocity of the particle and V_w = electroendosmotic velocity of the medium. In other words, the flow of water along the wall should be 50 per cent faster than the migration of the particle. The fact that cataphoretic velocity is independent of the shape of the particle does not indicate that the relationship for electroendosmotic and cataphoretic velocity is incorrect. (The equations were derived for a cylinder of infinite radius.) Furthermore, van der Grinten²¹ has claimed to have found the relationship mentioned. He used an open glass cell, and powdered glass made from the same glass as the cell itself. His method was open to several objections, the most important of which are (1) The use of an open system to determine cataphoretic velocity. (2) The presence of convection currents in the cataphoresis cell. (3) The absence of any evidence that the surface and particle had identical surfaces. The theory of Debye and Hückel and of Hückel seemed worthy of further investigation particularly in view of the data just presented.

Northrop and Kunitz²² have described an excellent three piece cataphoresis apparatus in which accurate and chemically clean experiments may be made. The apparatus was so modified that it was constructed of one piece of glass.* Its construction was simplified so that it could be rinsed with cleaning mixture rapidly and rapid measurements

²⁰ Freundlich, "Kapillarchemie," Leipzig, 1923, p. 559.

²¹ van der Grinten, *J. Chim. Phys.*, 23, 14 (1926).

²² Northrop and Kunitz, *J. Gen. Physiol.*, 7, 729 (1925).

* This cell will be described in detail in a future article in the *Journal of General Physiology*.

in a relatively ideal system were available. The results to be reported here are preliminary.

The movements of the particles in such a cataphoresis cell are those which would take place in a closed system (Fig. 3). Even with potential drops as high as 40 volts per cm. (in the case of non-electrolytes) there are no convection currents. Particles which are moving cataphoretically so quickly that their speed is too fast to be measured come to a complete and sudden stop on breaking the current. The measurements

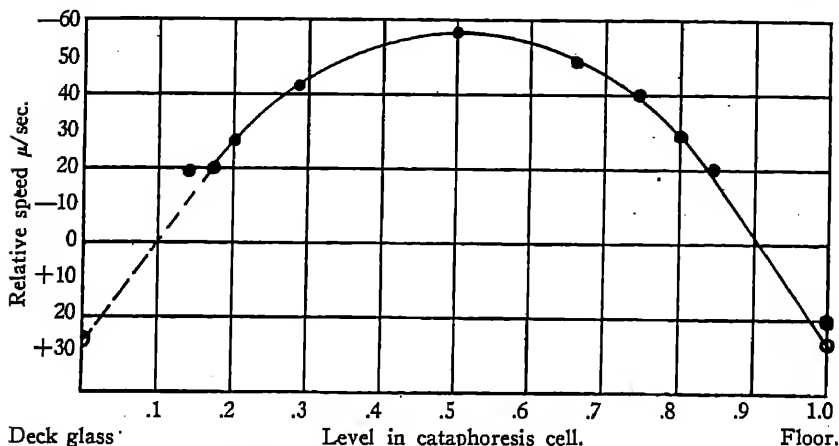


FIG. 3.—This gives the typical velocity-level curve found for cataphoresis cells of the type used in the experiments of this communication. The theory of von Smoluchowski is followed by such cells. The open circles are calculated velocities. The dots are observed velocities. From such curves the ratio V_w/V_p may be easily determined. (See text for references.)

of electroendosmosis along the wall of the cell may therefore be taken as representing the movement due to the difference of potential. Otherwise, as in the open cell used and described by van der Grinten, convection currents may destroy the accuracy of the measurements. (Van der Grinten used a pole-changer to annul these convection currents.) It was necessary in order to obtain reproducible results that cleaning solution have no effect on the glass (Pyrex) wall of the cell in successive measurements. This was found to be so. And powdered glass, made by pulverizing a piece of glass tubing from which the cataphoresis cell had been blown also preserved its surface qualities as determined by cataphoresis whether washed with distilled water or HCl or cleaning solution. Both particles and cell were, of course, washed well with distilled water before an experiment was begun. The cell was washed with cleaning solution before each refilling. The precautions used in

rinsing employed by Lachs and Kronman²³ when cleaning solution was used were found unnecessary.

TABLE III. *Cataphoretic Velocity of Powdered Glass in Distilled Water.**
Series I. (Magnification of 560.)

Date 1927	Experiment No.	Description	V_p $\mu/\text{Sec.}$	V_w $\mu/\text{Sec.}$	$\frac{V_w}{V_p}$	Remarks
Dec. 16	1	Particles unwashed in distilled water	a) 70	a) 179	a) 2.5	Note that by giving the particles and wall similar surfaces the ratio 1.5 is approached.
			b) 30	b) 96	b) 3.2	
			c) 12	c) 36	c) 3.0	
	2	Particles in $M/100$ $\text{AlCl}_3 + 2 \times 10^{-3}$ gelatin + 3 drops acetic acid	a) 21	a) 30	a) 1.43	
			b) 3.1	b) 4.4	b) 1.42	
Dec. 18	3	Particles unwashed in distilled water	30	96	3.2	
	4	Particles after cleaning solution-water (1:1) at 60°C.	30	84	2.8	Note again the ratio V_w/V_p in the neighborhood of 3.0, the absence of effect of strong acids on glass wall and particles. In this series (3-7) the $P.D./\text{cm}$ was the same.
	5	Particles after washing with distilled water	27	80	3.0	
	6	Particles after cleaning solution in distilled water 48 hours	30	92	3.0	
	7	Particles washed with distilled water	32	94	2.9	
Dec. 19	8	Particles + $M/50$ acetic acid	32	66	2.0	
	9	Particles in $M/50$ acetic acid + 2×10^{-3} egg albumin	a) 22	a) 32	a) 1.46	
			b) 44	b) 56	b) 1.27	
			c) 41	c) 60	c) 1.46	
			d) 21	d) 35	d) 1.67	

* The experiments described in the table show that the cataphoretic velocity, V_p , of powdered glass suspended in distilled water is about $\frac{1}{3}$ that of the speed of the water, V_w , against the glass wall of the cataphoresis cell made from the same glass. Coating both particle and wall with an adsorbed layer of protein gives values V_w/V_p near 1.5. This corresponds to the ratio predicted by the theory of Debye and Hückel. The values in the table are relative for each experiment. In experiments with sub-letters, a, b, c , etc., the particles were resuspended by turning the cell upside down so that different applied e.m.f.'s could be employed. The apparatus was washed with cleaning mixture before each experiment.

V_w is easily calculated from the speed of the particles. It can be shown that $V_w = 2V_p$, where V_p is the speed of the water in the middle of the cell. These data show that van der Grinten's values may or may not be a correct expression of the ratio $\frac{V_w}{V_p}$.

²³ Lachs and Kronman, *Est. Bull. Acad. Pol. Sci. Lettres A*, 1925, p. 289.

Series II. (Magnification of 1120.)

Date 1927	Experi- ment No.		V_p $\mu/\text{Sec.}$	V_w $\mu/\text{Sec.}$	$\frac{V_w}{V_p}$	Remarks
Dec. 20	1	Particles in distilled water	a)18 b)22	a)54 b)49	a)3.0 b)2.2	
	2	Particles + M/50 acetic acid	a)23 b)25	a)45 b)38.5	a)1.9 b)1.55	
	3	Particles in M/50 acetic acid + 2×10^{-3} egg albumin	a)14 b)33	a)23.5 b)42	a)1.7 b)1.25	Suspension of the particles in acetic acid alone also causes lowering of the V_w/V_p ratio.
	4	Particles in distilled water	a)36 b)20	a)98 b)52	a)2.7 b)2.6	See experiment 6 also.
	5	Particles washed with distilled water	a)30 b)18.5	a)76 b)55	a)2.5 b)2.9	
	6	Particles in M/50 acetic acid	35	52	1.5	
	7	Particles in M/10 acetic acid + 10^{-3} gelatin	30.5	45	1.45	

Mean $\frac{V_w}{V_p} \left\{ \begin{array}{l} (1) \text{ particles in water} = 2.85 \\ (2) \text{ particles and wall covered by protein films} = 1.46 \end{array} \right.$

The most significant results thus far obtained are given in Table III. In distilled water the velocity of the particle is about one-third that of the velocity of water against the glass of the cell. In this respect van der Grinten's results have not been duplicated. This could be due however to differences arising in the surface of the glass powders. The stresses incidental to blowing and pulverizing could have produced structural changes leading to the difference here noted. On coating the glass of the cell and of the particles with a layer of adsorbed protein,* however, the ratio V_w/V_p dropped to about one half that observed for the clean glass surface. And the mean of four determinations of this ratio is 1.46 which is remarkably close to the ratio 1.5 demanded by the theory of Debye and Hückel. (Suspending the particles in fairly strong solutions (M/50) of acetic acid also change the V_w/V_p ratio.) From these data the conclusion could be drawn that the factor $1/6\pi$ is valid for the cataphoresis of particles of any shape; and the factor $1/4\pi$ is valid for electroendosmosis against a flat surface. The hydrodynamic conditions existing in this cataphoresis cell, however, are too ill defined to permit of any conclusions with one exception. We do not know at present what the ratio V_w/V_p is for such conditions as examined here and by van der Grinten. Experiments in progress performed with a

* It will be shortly demonstrated that glass or quartz surfaces adsorb protein from dilute solutions and have surfaces which behave like the protein adsorbed.

flat surface in a cataphoresis cell more uniform in cross section have given values of V_w/V_p very close to 1.0. In the case of endosmosis through a membrane made up of a powder, on the other hand, V_p/V_w should be equal to 1.0.

THE MIGRATION OF INERT PARTICLES AND BLOOD CELLS IN GELATIN SOLS AND GELS ^{10, 6, 18}

It has already been mentioned that the cataphoretic velocity of microscopic particles in serum was proportional to the drop in potential. (Serum, however, belongs to that group of fluids that follow Poiseuille's law.*) Just what influence a liquid having plastic flow would have on the mobility of these particles was uncertain. The following experiments were performed. The mobility of zinc dust and of quartz particles was studied in a 1 per cent Agfa gelatin solution (at 20° C.) at intervals from ½ hour to 5 hours after preparation. During this time the transformation from the sol to the gel takes place. Particles which fall to the bottom of the cell after about 2 hours are well supported by the rigidity produced by the "ageing" of the sol. As Table IV shows, in such sys-

TABLE IV. *Influence of Medium on Cataphoresis of Zinc Particles at Room Temperature.*

Age of Sol or Gel in Hours	Character of Medium	In μ /Sec. for $\frac{1 \text{ Volt}}{\text{Cm.}}$
0.5.....	Sol	0.36
1.0.....	Sol	0.35
2.0.....	Plastic sol	0.35
3.0.....	Plastic sol	0.34
3.5.....	Soft gel	0.33
5.0.....	Gel	0.36

Zero time is ½ hour. See Table Vb.

tems the mobility of the particles mentioned is the same in the 5 hour gel as it is in the ½ hour sol which has practically viscous flow. It is most interesting to see particles of zinc, 25 μ in diameter, which remain supported by the gel at the same level in the cell for hours, move under the influence of an electric field as if they were in a liquid having a small coefficient of viscosity. The mobility was proportional to the drop in potential between 1 volt and 70 volts per cm. and remained the same in consecutive measurements. Particularly striking is the behavior of large air bubbles which remain suspended in the plastic sol or gel.

Air bubbles move with the same speed as the other particles. There is no deformation. The movement of the particles and air bubbles just described is not an independent migration. They are surrounded by

* This is true only for serum that is not fresh. Fresh serum may show a slight deviation.

gelatin films, and migrate with the same speed as the gelatin micellae themselves. The air bubbles investigated by McTaggart²⁴ have shown similar properties in regard to ions.

The movement of the particles at different levels in the cell is precisely the same as that found for particles suspended in water, the velocity curve of the particles at different levels has the same shape as that in Figure 3. This means that there is shear taking place between different levels of the sol or gel. The tearing, however, is insufficient to cause the sol or gel to become fluid enough to permit the particles from falling to the floor of the cell. In these soft gels, then, microscopic particles behave somewhat similarly to the soap micellae and intra-micellae fluid of McBain's soap gels. It has not yet been demonstrated that particles of the order $6\ \mu$ can migrate through a gel. This will be shown shortly.

In more concentrated gels the mobility is still proportionately very high when compared to the stiffness of the gel.²⁵ The shear occurring during cataphoresis of the micellae of the gelatin produces, however, a softening of the gel with an increase in speed of the particles. In Table Va is a typical example. The gel rebuilds itself in a very short time. This is a case of the reversible gel-sol transformation which Freundlich^{25, 26} has called thixotropy. Here, the thixotropy has not been invoked by the usual mechanical method of shaking, but by the shear following water flow caused by electrical differences of potential. Gelatin gels show the same phenomenon when shaken.²⁵

It was of interest to follow the changes in viscosity η , and apparent viscosity, η' , (in the Hess viscosimeter) simultaneously with the meas-

TABLE Va. *Thixotropy of a 2 Per Cent Gelatin Gel Due to Cataphoretic Shear and Demonstrated by Increasing Cataphoretic Velocity.*

To and Fro Migration Number	Time to Migrate 50 μ . Sec.	To and Fro Migration Number	Time to Migrate 50 μ . Sec.	Remarks
1.....	40	12.....	14	
2.....	35	14.....	12.4	
3.....	33.5	16.....	11.5	This gel rebuilds itself rapidly and the experiment may then be repeated.
4.....	30	18.....	11.6	
5.....	26	20.....	10.5	
6.....	22.5	22.....	10.2	
7.....	20	24.....	9.8	
8.....	18	26.....	10.2	Particles remain suspended.
9.....	16.5	27.....	10.1	
10.....	16			

Age of gel $2\frac{1}{2}$ hours. Quartz particles. Current .003 amperes. Temperature 20°C .

²⁴ McTaggart, *Phil. Mag.* (6), 27, 297 (1914); 28, 367 (1914).

²⁵ Freundlich and Abramson, *Z. physik. Chem.*, 131, 278 (1928).

²⁶ Freundlich and Rosenthal, *Z. physik. Chem.*, 121, 463 (1926).

TABLE Vb. *Absence of Thixotropy in a 1 Per Cent Gel.*

Quartz powder.

To and Fro Migration Number	Time to Migrate 100 μ . Sec.	To and Fro Migration Number	Time to Migrate 100 μ . Sec.	Remarks
1.....	17.0	7.....	17.1	
2.....	16.8	8.....	16.8	
3.....	17.0	9.....	16.5	
4.....	16.7	10.....	16.5	
5.....	16.7	11.....	16.7	
6.....	16.5	12.....	17.0	Particles remain suspended.

Age of gel 24 hours. Temperature 20°C.

urements of cataphoresis in the case of the 1 per cent gel. Figure 4 shows that during sol-gel transformation at the times when the mobility

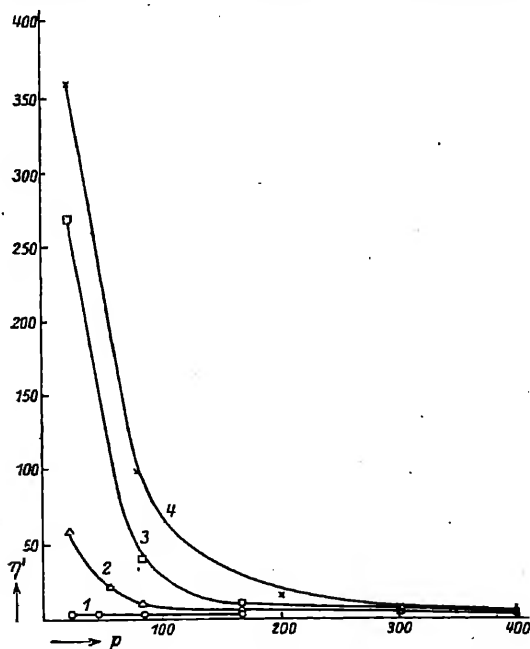


FIG. 4.—During the ageing of a 1 per cent gelatin gel η' , the apparent viscosity, increases appreciably. (Curve 4 is $3\frac{1}{2}$ hr. gel.) Where the curves run parallel to the abscissa, η , the true viscosity is measured. These curves demonstrate, then, that the true viscosity is changed relatively slightly, if at all, during gelation.

measurements were made, η' increased enormously with low rates of shear. With sufficiently high rate of shear, values obtained for η' ap-

proached η , the true viscosity asymptotically. (The same has been found for dibenzoylcytosteine and sodium oleate gels.) This is compatible with the constant cataphoretic velocity during the sol-gel transformation.

That a microscopic particle may migrate through a gel practically independent of the gel structure is shown in Table VI. In a fresh,

TABLE VI. *The Cataphoresis of Red Cells, Leucocytes and Quartz Particles in a 1.2 Per Cent Gelatin-Serum Gel.*

Nature of Medium	Relative Velocity			Remarks
	Red Cells μ per sec.	Leucocyte μ per sec.	Quartz μ per sec.	
Fresh sol (slightly plastic)	12	6	7	The differences between quartz particles and leucocytes are actually less when more accurate determinations are made.
40 minutes later (soft gel)	11	5	6	The slight decrease in velocity is due to the fact that these values are means of several measurements where the first speeds were lower due to the presence of a slightly stiffer gel, which became softer during the measurements.

The measurements given here are relative values for the level of the electrophoresis cell where the speed of the water is negligible. Note the independent migration of the red cells in sol and gel.

slightly plastic 1.2 per cent gelatin-serum sol (the particles settled out slowly), the red cells migrated twice as fast as the quartz particles and white cells in the same suspension. Forty minutes later a soft gel had formed. (The cells remain suspended in such a gel and they may be studied for hours.) In this soft gel the red cells still maintained their independent movement and had an absolute velocity not appreciably lower than had been found in the serum. Particles like red cells, which do not coat themselves with the protein of the gel are forced by the potential gradient through such a soft gel practically as if no gel structure were present.

In stiffer gels all particles moved at first with the gel and therefore with the same velocity. They are thus held by the more rigid gel structure. Moving the particles back and forth (the gel is broken up simultaneously) produced a softening of the gel with increasing velocity of the particles, the red cells rather suddenly assuming their characteristic independent velocity. If the experiment be discontinued at this point, the gel reforms in a short time and the experiment may be repeated with the same cells which remained suspended at the same level in the cell. When the circuit was broken in stiff gels, the particles moved back a short distance. This phenomenon is similar to that found

by Freundlich and Seifriz²⁷ in the case of nickel particles drawn through plastic systems by a magnetic force. In fibrin gels, the migrating particles were hindered or totally arrested in their movement by the fibrin network.

The fact that microscopic particles may migrate through certain gels with the utmost ease is most significant.^{4, 6} It suggests that the movements of cells through gel-like systems, *e.g.*, the emigration of the leucocyte through the capillary wall, could occur with facility in the presence of small electromotive forces. If there is a difference of potential in the membrane of the capillary wall of 1 millivolt (with the assumption that the negative stream is toward the point of injury) and if the thickness of the wall is $.5 \mu$, the drop in potential is 20 volts per cm. This electromotive force is of a sufficient order of magnitude, as are those calculated for the connective tissue, to bring a white cell cataphoretically to the point of injury within the time required for this process to occur.

THE ADSORPTION OF PROTEINS BY QUARTZ AND GLASS

It has been previously mentioned that quartz and glass adsorb proteins from dilute solution. There is much related literature dealing with surfaces other than quartz. The early observations of Zsigmondy²⁸ after those of Lottermoser and Meyer²⁹ have been followed by the experiments of Whitney and Blake,³⁰ Walpole,³¹ Brossa and Freundlich³² and Pütter.³³ More recently the work of Loeb,³⁴ of Davis,³⁵ of Northrop and De Kruif³⁶ and of Hitchcock³⁷ have related the phenomena more intimately with the isoelectric point of the protein. The experiments on the cataphoresis of quartz particles differ from previous studies and represent an advance as the experiments can be performed in chemically clean systems (with the modified Northrop-Kunitz³¹ cataphoresis cell) on the fairly well defined and easily reproducible surfaces of quartz and pyrex glass. They differ somewhat from the experiments of Loeb with collodion particles, but his experiments and those of Walpole furnish qualitative evidence that inert particles in dilute protein solutions have films of the protein which characterize the surface of the particles. The curves (Fig. 5) demonstrate that quartz particles adsorb protein on both sides of the isoelectric point from gelatin solu-

²⁷ Freundlich and Seifriz, *Z. physik. Chem.*, 104, 233 (1923).

²⁸ Zsigmondy, *Z. anal. Chem.*, 40, 697 (1901).

²⁹ Lottermoser and Meyer, *J. prakt. Chem.*, 56, 242 (1897); quoted by Freundlich, "Kapillarchemie," 1923, p. 808.

³⁰ Whitney and Blake, *J. Am. Chem. Soc.*, 26, 1339 (1908).

³¹ Walpole, *Proc. Physiol. Soc.*, Oct. 18, 1913.

³² Brossa and Freundlich. See Freundlich, "Kapillarchemie," Leipzig, 1923, p. 799 et seq.

³³ Pütter, *Z. Immunitäts.*, 32, 538 (1921).

³⁴ Loeb, *J. Gen. Physiol.*, 6, 116 (1923).

³⁵ Northrop and De Kruif, *J. Gen. Physiol.*, 4, 639 (1922).

³⁷ Hitchcock, *J. Gen. Physiol.*, 8, 61 (1925).

tions whose initial content of protein was 10^{-7} grams per cc. At initial concentrations of about 10^{-5} grams per cc. of protein the quartz particles act as if they were particles of the protein itself. Advantage was taken of the fact that Svedberg and Tiselius³⁷ have determined the mobility of *native* egg albumin (by studying the boundary movement with ultra-

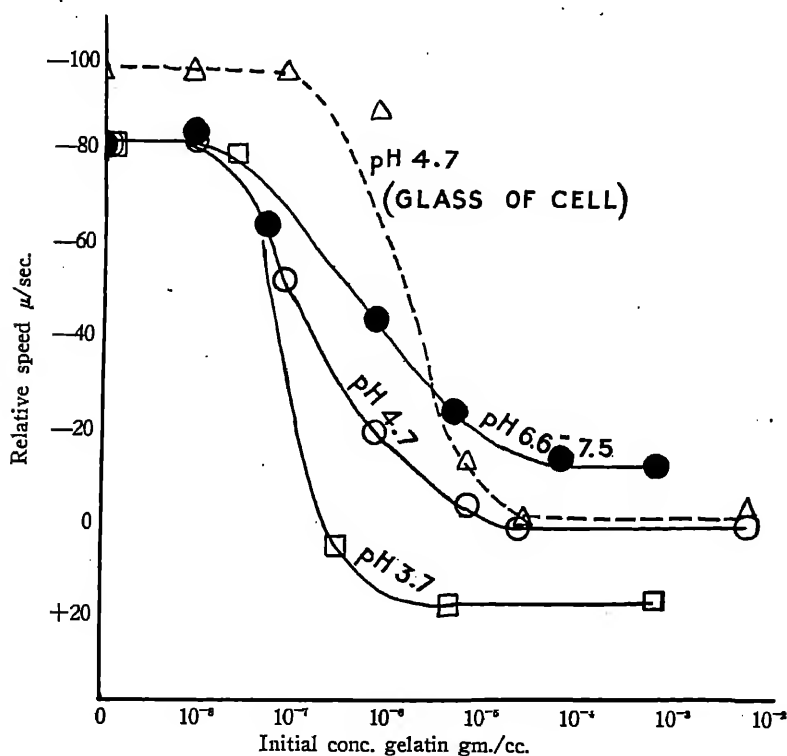


FIG. 5.—The adsorption of gelatin by quartz on both sides of the isoelectric point of gelatin. The dotted line shows the same change taking place simultaneously on the glass wall of the cataphoresis cell at pH 4.7. Note that at concentrations of gelatin less than 10^{-4} gm. per cc. the quartz particles act like particles of the protein itself.

violet photography) to devise a method for the study of protein mobility based on the adsorption of proteins by quartz. The curve in Figure 6 obtained from dilute solutions (10^{-8} grams/cc.) of protein with suspended quartz particles agrees with the data given by Svedberg and Tiselius. Measurements may be made immediately after suspending

³⁷ Svedberg and Tiselius, *J. Am. Chem. Soc.*, 48, 2272 (1926).

the particles in the protein solution. The cemented cell may be used for such determinations. This method furnishes an approach to the study of protein mobility and the properties of proteins as they probably frequently exist on the surface of the living cell. The leucocytes have

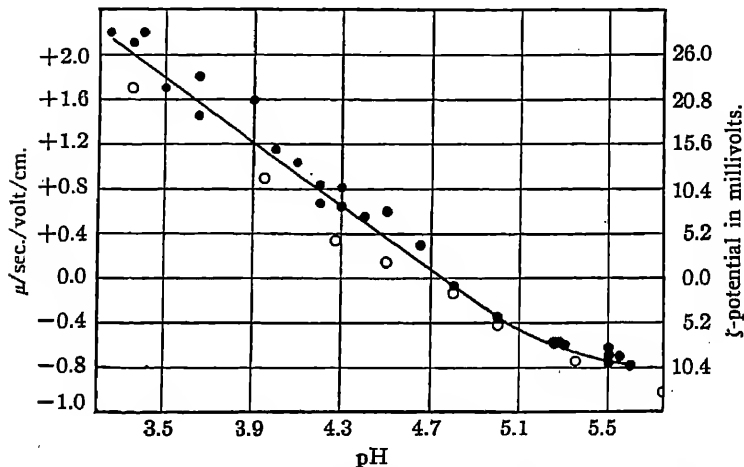


FIG. 6.—The dots indicate protein mobility found in the experiments described in this communication. The open circles are the recalculated data from Svedberg and Tiselius. The signs on the ordinate refer to the charge.

been shown to migrate with the same speed as quartz with an adsorbed protein film. Shibley⁸⁸ had shown the same phenomenon for sensitized bacteria and denatured globulin.

*Kaiser Wilhelm Institute for Physical Chemistry
and Electro-Chemistry,
Berlin-Dahlem, Germany,
and
Johns Hopkins Medical School,
Baltimore, Md.*

⁸⁸ Shibley, *J. Exp. Med.*, 40, 453 (1925); 44, 667 (1926).

METHODS OF STUDYING THE SURFACES OF LIVING CELLS, WITH ESPECIAL REFERENCE TO THE RELATION BETWEEN THE SURFACE PROPERTIES AND THE PHAGOCYTOSIS OF BACTERIA

BY STUART MUDD, BALDUIN LUCKÉ, MORTON MCCUTCHEON, AND
MAX STRUMIA

INTRODUCTION

Direct study of the wetting properties, of the electrokinetic potential difference, and of the cohesiveness of suspensions of living cells has been made possible by recent technical advances. In the experiments here reported these several properties of living cell surfaces have for the first time been studied together and related to the important biological phenomenon of phagocytosis.

The white cells (leucocytes) of the blood ordinarily take up (phagocytize) bacteria only to a slight degree. When, however, the bacteria have been treated with fresh normal blood serum in high concentration, or with specific immune blood serum either in high or low concentration, phagocytosis is greatly increased. The substances in or properties of serum which cause this increased phagocytosis are called *opsonins* in the case of normal serum, *bacteriotropins* in the case of immune serum. Both are important factors in defense against infectious disease.

The present experiments represent the first stage of a study directed toward analysis of the mechanism of opsonin and bacteriotropin action in physical-chemical terms. They are intended to answer the question: "What changes do sera effect in bacteria in preparing them for phagocytosis?"

The bacteria used have belonged to the so-called "acid-fast" group, of which the tubercle bacillus is the best known species. The surfaces of these bacteria have been shown to contain protein¹ and lipoid² and not improbably also carbohydrate components, and they are ordinarily taken up only to a slight degree by leucocytes.

Immune sera have been prepared by injecting rabbits with *Bacillus tuberculosis* and a variety of related microorganisms. The bacteria, treated with serial dilutions of normal and immune sera, have been studied in the following reactions: (1) The bacteria remain in serum

¹ Freund, *Am. Rev. Tuberculosis*, 12, 124 (1925).

² Mudd and Mudd, *J. Exper. Med.*, 46, 167 (1927).

dilutions overnight and the degree of precipitation or agglutination is then read in each tube. (2) The serum-bacterial mixtures are strongly centrifugated and the sediments resuspended by shaking³ until the untreated control tubes show an even suspension; the treated sediments resuspend in flocculi increasing in coarseness with their cohesiveness. (3) The treated bacteria are washed and their wetting or interfacial tension properties are then estimated in the interface reaction;^{2, 4} the bacteria are observed microscopically in an oil-water interface; before treatment they pass readily into the oil, after treatment they resist passing into the oil in proportion to the degree of their interaction with serum. (4) The electrokinetic potential difference between the washed bacteria and their suspending medium is calculated from determinations in a microcataphoresis cell. These four reactions together give a picture of the surface properties of the bacteria. (5) Meanwhile mixtures of rabbit leucocytes with, (a) serum-treated, washed bacteria, or with (b) bacteria and serum dilutions, are rotated in stoppered vials on a Robertson agitator.⁵ Smears are made from each mixture, stained, and a hundred leucocytes in each smear are observed microscopically. The per cent of leucocytes which have taken up bacteria is recorded.

The several reactions alluded to will now be described in more detail both for the purpose of clarifying the present data and because these methods are capable of manifold application in the study of other suspensions and emulsions, the action of dispersing agents, and so forth.

REACTIONS

AGGLUTINATION

The agglutination reaction consists in mixing given volumes of bacterial suspension with serial dilutions of serum and allowing several hours of contact. If interaction has occurred, the bacteria in the higher serum concentrations will be in some degree flocculated and sedimented. Agglutination decreases and finally disappears with diminishing serum concentrations.

Serum agglutination is a two-stage reaction.⁶ In the first stage serum components are deposited on the bacteria, combining chemically with substances in the bacterial surface, reducing the electrokinetic potential difference and increasing cohesiveness.⁷ In the second stage the bacteria, "sensitized" by the serum components, are flocculated.

² Mudd, *J. Immunol.*, 13, 113 (1927).

³ Mudd and Mudd, *J. Exper. Med.*, 46, 173 (1927).

⁴ Robertson, Woo, and Cheer, *J. Exper. Med.*, 40, 487 (1924).

⁵ Bordet, *Ann. Inst. Pasteur*, 13, 225 (1899).

⁶ Northrop and DeKruif, *J. Gen. Physiol.*, 4, 639, 655 (1922); Shibley, *J. Exper. Med.*, 40, 457 (1924); 44, 667 (1926); Freund, *Am. Rev. Tuberculosis*, 12, 124 (1925); Falk and Jacobson, *J. Infectious Diseases*, 38, 182 (1926); Mudd and Mudd, *J. Exper. Med.*, 46, 173 (1927).

Agglutination is one of the chief reliances of bacteriologists in studying the relationships of the component materials of bacteria and other cells.⁸ Jones has lately shown that this reaction may be used to identify various proteins adsorbed on collodion particles or bacteria.⁹

RESUSPENSION

Under various special conditions agglutination as ordinarily carried out is unsatisfactory. To meet these cases a modified agglutination or "resuspension" reaction has been developed.¹⁰ After the bacteria have been in contact with the serum dilutions and the agglutination readings have been made in the usual way, all tubes are centrifugated at high speed until the bacteria are completely sedimented or practically so. The supernatant fluid is decanted and two drops of 0.85 per cent NaCl solution are added to the bacterial sediment in each test tube. The tubes are arranged in a rack with the control tubes, *i.e.*, tubes in which the bacteria had been mixed with 0.85 per cent NaCl solution without serum, in the middle. The rack is now shaken uniformly until the sediment in the control tubes is just brought into even suspension. The bacteria which have been treated with serum may resuspend in flocculi whose coarseness increases with the concentration of serum and with the affinity of the components of the serum ("antibodies") for the particular bacteria used.

This reaction has the advantage of eliminating certain imperfectly controlled variables in the second stage of the agglutination reaction. The bacteria, after adsorption of serum components, are forcibly pressed together by centrifugal force. Their subsequent resuspension depends primarily upon and gives a roughly quantitative estimate of their cohesion.

INTERFACE REACTION

The test particles are directly observed in the boundary surface between two immiscible or partially immiscible liquids. In the present experiments the bacteria are observed microscopically with the aid of a dark-field illuminator in the liquid-liquid boundary of a two-phase film composed of tricaprylin and of an 0.85 per cent solution of NaCl in water. The oil slowly encroaches on the water; the interface, therefore, successively overtakes the suspended or sedimented bacteria and

⁸ Wells, "The Chemical Aspects of Immunity," New York, The Chemical Catalog Co., Inc., 1925, ch. VI.

⁹ Jones, *J. Exper. Med.*, 46, 303 (1927).

¹⁰ Gaechtens, W., *Münch. med. Wochschr.*, 53, 1351 (1906); *Arch. Hyg.*, 66, 1351 (1908); Gates, *J. Exper. Med.*, 35, 63 (1922); Mudd, *J. Immunol.*, 13, 113 (1927).

their degree of resistance to passage from the aqueous to the oil phase may be observed in a roughly quantitative way. The surfaces of acid-fast bacteria contain much non-polar material;² these microorganisms pass, correspondingly, easily or even spontaneously into the non-polar phase. After interaction with serum the bacterial surface is coated partially or completely with polar serum components, and is correspondingly resistant to passage into the non-polar liquid. This change constitutes a positive interface reaction.⁴

The resistance of the bacterial clumps to dispersion by the tension of the interface can also be directly observed in this reaction, and a second rough estimate of bacterial cohesion is thereby gained.

The wetting properties observed in this reaction are obviously dependent upon interfacial tension relations. Consider a solid particle in the boundary surface between water and an organic liquid. The particle will so dispose itself that at equilibrium:

$$T_{so} = T_{sw} + T_{ow} \cos. \theta.$$

where T_{so} is the tension at the solid-organic phase interface, T_{sw} is the tension at the solid-water interface, T_{ow} is the liquid-liquid tension and θ is the contact angle between the aqueous and solid phases at any point O on the surface of the solid particle.

The necessary conditions for the equilibrium of the particle in the boundary surface are that $T_{so} < T_{sw} + T_{ow}$ and $T_{sw} < T_{so} + T_{ow}$.

If $T_{so} > T_{sw} + T_{ow}$, the interface will be displaced toward the organic side until the particle is fully wetted and surrounded by the watery phase. If $T_{sw} > T_{so} + T_{ow}$, the particle is wetted and surrounded by the organic phase.

Applications of the technique to the study of various cells under a variety of conditions have been described by Mudd and Mudd.^{2, 4, 11} A technique modified for use in gross has been described by Mellon.¹² Many applications in colloid chemistry are obviously possible.

CATAPHORESIS

The electric potential difference between bacteria and their suspending medium is calculated by the Helmholtz-Lamb formula from determination of cataphoretic velocity in the microcataphoresis cell of Northrop and Kunitz.¹³ * After the agglutination and resuspension read-

¹¹ Mudd and Mudd, *J. Exper. Med.*, 40, 633, 647 (1924); 43, 127 (1926); *Biochem. Z.*, 186, 378 (1927).

¹² Mellon, *Proc. Soc. Exp. Biol. Med.*, 23, 716 (1926).

¹³ Northrop and Kunitz, *J. Gen. Physiol.*, 7, 729 (1924-25).

* The cell used in these experiments was made by J. D. Graham, glassblower at the University of Pennsylvania, by the following method. The top and bottom of the cell are formed by thin microscopic slides; these are held 0.6 to 0.8 mm. apart by narrow

ings have been made, bacteria are suspended in a large excess of 0.85 per cent NaCl solution and again centrifugated; the supernatant is decanted and the sediment shaken up in fresh NaCl solution. Some of this washed suspension is used for the interface reaction and some for cataphoresis. The cataphoresis cell is mounted over a dark-field condensor. Three readings are made at each of three levels, namely at one, three and five-twelfths of the distance from the bottom to the top of the inside of the cell. The algebraic mean of the rates at these levels is used.

PHAGOCYTOSIS

The phagocytic cells used were rabbit polymorphonuclear leucocytes. They were obtained by injecting about 200 cc. of sterile 0.9 per cent NaCl solution intraperitoneally in a rabbit;¹⁴ after 3 to 4 hours about 80 to 100 cc. of the fluid, now containing leucocytes, were recovered by peritoneal puncture. The fluid was received in a flask containing 0.7 per cent NaCl and 1.1 per cent Na citrate in the proportion of 3 parts of peritoneal fluid to 1 part of citrate solution. The leucocytes were centrifugated, washed and resuspended in 0.9 per cent NaCl solution. Into each of a series of small hardglass vials of uniform size there were distributed 0.1 cc. of serum dilution, 0.1 cc. of bacterial suspension and 0.2 cc. of the washed leucocytic suspension. The vials were stoppered with freshly paraffined corks and immediately placed on a Robertson⁶ rotating machine. All vials were rotated at 4 r.p.m. for 15 minutes at room temperature ($25 \pm 1.5^\circ \text{C.}$). The vials were then quickly placed in racks and plunged in ice water to stop phagocytosis. Spreads were made from each vial and stained. The numbers of leucocytes that had ingested bacilli were determined by observing microscopically 100 cells (sometimes 200 cells). Each of two observers counted 50 (or 100) cells; the results agreed very closely.

RESULTS

Sera reacting with bacteria of the tubercle bacillus group have been shown by the above reactions to cause agglutination, increased cohesion

glass strips cut from similar slides. The margins of the upper and lower slides and the glass strips between them are pressed together by a specially constructed metal clamp and are fused by a hot, minute, pointed flame. The cell is then completed by fusing glass tubing onto its two ends and sealing-in the platinum electrodes between the flat portion of the cell and its end tubes. The open ends of the glass tubes are ground plane so that a glass to glass contact can be made inside the rubber tube connecting with the Zn electrode vessels. This modified method of making the Northrop-Kunitz cell is due to Dr. M. Kunitz. Its advantages are in giving a cell of uniform depth and width and in eliminating optical distortion due to waviness in the drawn glass. The same cell can be adapted to use with the slit ultramicroscope by plane-polishing one edge so as to allow a horizontal beam of light to enter the cell.

¹⁴ Hamburger, in E. Abderhalden's "Handbuch der biologischen Arbeitsmethoden," Abt. IV, Teil 4, Heft 3, 1926, p. 953.

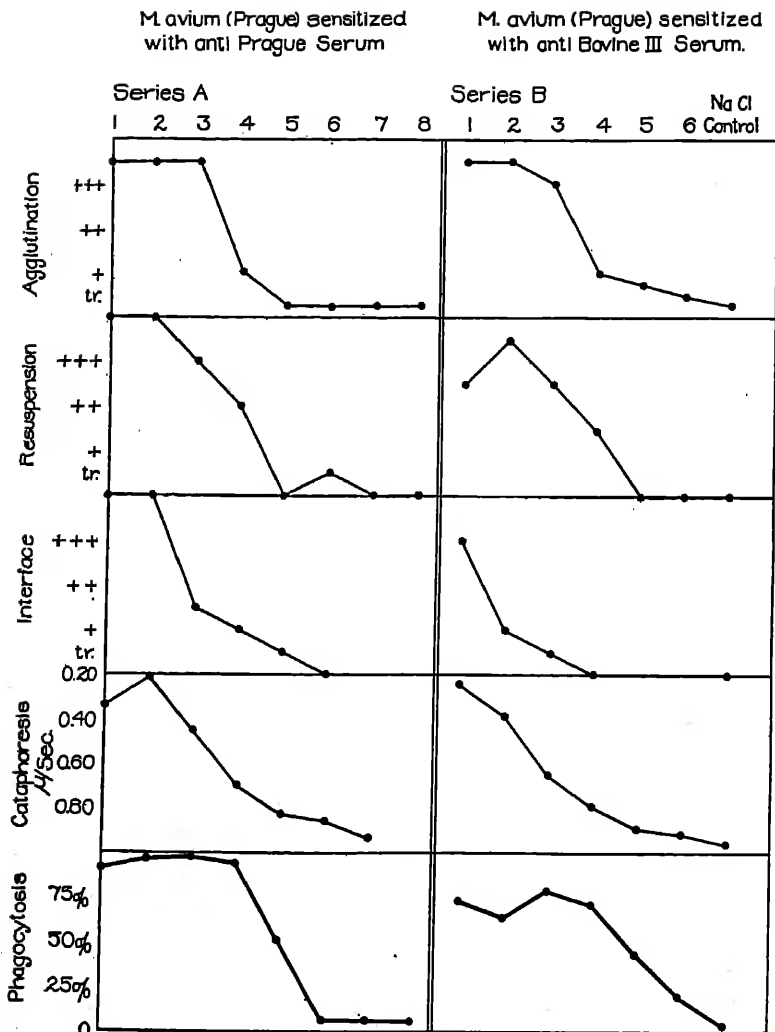
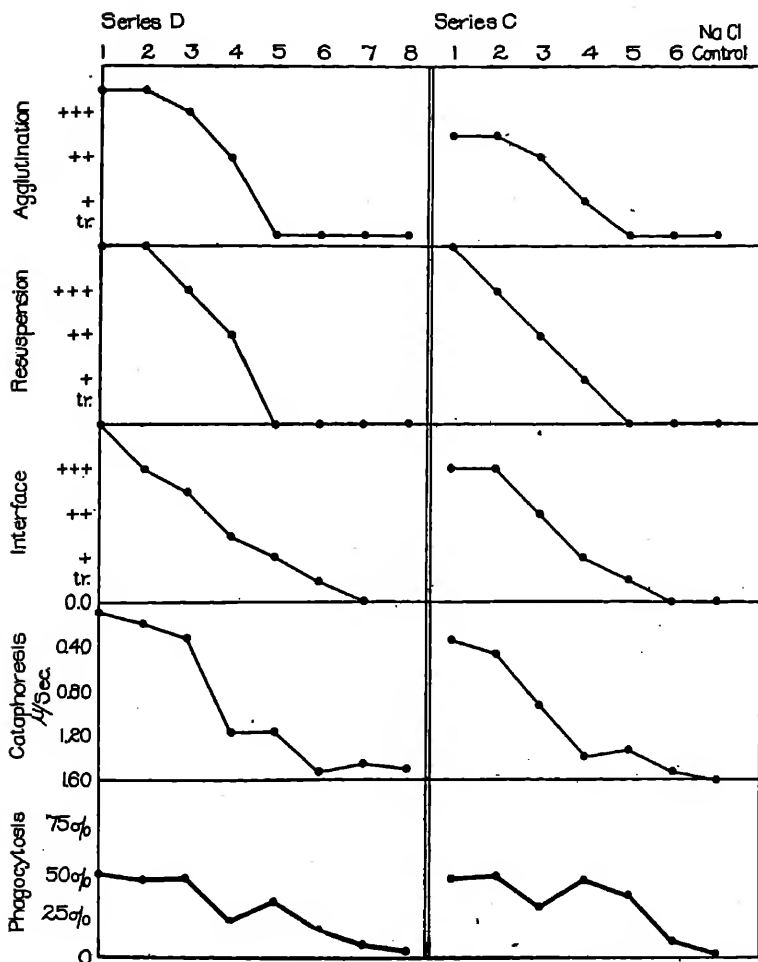


FIG. 1.—Reactions between an avian and a bovine strain of tubercle bacillus and of the several reactions. Abscissæ are dilutions of the serum used to sensitize dilution of 1:16, 3 of 1:64, 4 of 1:256, etc. It is seen that the changes in

M. tuberculosis (Bovine III) sensi-
tized with anti Bovine III Serum.

M. tuberculosis (Bovine III) sensi-
tized with anti Prague Serum.



the sera of rabbits immunized to these two strains. Ordinates are the intensities the bacteria, expressed as powers of four. Thus 1 is a dilution of 1:4, 2 is a surface properties and in phagocytosis are in general parallel.

of the bacteria, reduced surface potential difference, change from a bacterial surface readily wet by oil to a surface wet by water, and increased phagocytosis. The most important relationship thus far brought out is that in practically every instance in which an immune or normal serum has caused increased phagocytosis it has caused in approximately corresponding degree the above indicated changes in surface properties. Conversely when fresh unheated rabbit sera have reacted with bacteria so as to produce the surface changes, corresponding increase in phagocytosis has uniformly resulted.

An illustrative experiment is shown in Figure 1. The sera here used were taken from two rabbits injected with tubercle bacilli, respectively of the bovine and avian types. The corresponding bacilli were used as test suspensions. After treatment with the serum dilutions the bacteria were washed before use in the interface, cataphoresis and phagocytosis reactions.

Intensities in the first three reactions are plotted on the axis of ordinates as plus signs. Cataphoretic velocities are given in micra ($\text{cm.} \times 10^{-4}$) per second per volt/cm. fall in potential along the cell. If we assume¹⁵ the dielectric constant and coefficient of viscosity of the suspension of bacteria in 0.85 per cent NaCl solution to be those of pure water at 20° C. (assumptions which of course are not strictly accurate), each velocity given may be converted into electrokinetic potential difference in millivolts by multiplication by 12.6. Ordinates for phagocytosis are the percentage of leucocytes which contain bacteria. Abscissae in all cases are the serum dilutions in the final serum-NaCl solution bacterial mixtures.

The parallelism of the effects shown by the several reactions is very striking indeed for this type of work, which from its nature cannot attain to a high degree of precision. The degrees of interaction of each serum with its corresponding bacterial type and with the other type in this experiment do not differ greatly because of the close relationship of these two particular bacterial types. With less closely related species each serum reacts more strongly with its corresponding type than with other types; *i.e.*, serological "specificity" is shown, and is shown in all the reactions.

The sera of four rabbits have been followed by similar tests at weekly intervals during the course of active immunization by injection of bacteria. Striking correlation was found between the bacterial surface changes and the increase of phagocytosis produced by the sera of these animals. Data for one of these animals is given in Figure 2. Ordinate values in this figure are the highest dilutions of serum which gave clearly demonstrable effects in the several reactions. Abscissae are time intervals

¹⁵ Northrop and Cullen, *J. Gen. Physiol.*, 4, 638 (1921-22).

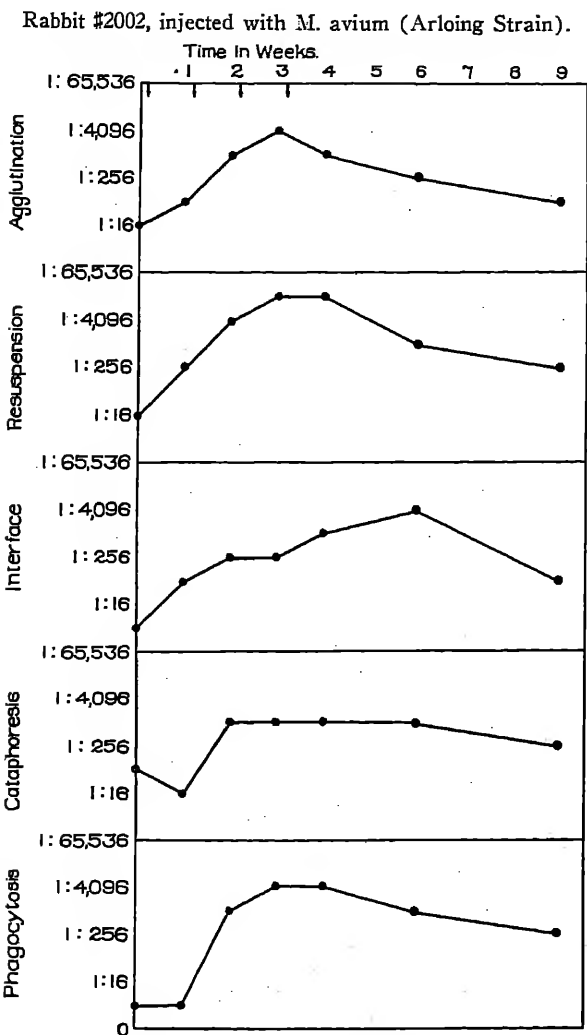


FIG. 2.—The course of immunization of a rabbit with a strain of avian tubercle bacillus. Four intravenous injections of bacilli at times indicated by vertical arrows. Ordinates are the highest dilutions of the rabbit serum which gave clearly demonstrable effects in each of the reactions. Abscissae are times in weeks. Correlation between surface properties and phagocytosis again apparent.

in weeks from the start of the experiment. Absolute values for the end points differ in the several reactions, as was to be expected; however, the general parallelism is again striking.

The sera of seven rabbits suffering from actual tuberculous disease have been similarly followed at weekly intervals. Again correlation between surface changes and phagocytosis has been excellent.

However, when bacteria have been treated with aged or heated sera, the surface changes have been produced in a number of instances in which phagocytosis has failed. Thus in five of twenty-seven series with immune sera aged for about sixteen to eighteen months, surface changes were produced but phagocytosis failed. Moreover in series with aged sera phagocytosis in some instances reached a maximum not, as with the surface reactions, in the highest serum concentrations, but in serum concentrations as low as one-tenth of one per cent. In nine out of sixteen series in the presence of heated normal rabbit serum perceptible surface change was elicited without increase in phagocytosis. Finally, bacteria treated with normal human sera failed to cause phagocytosis by rabbit leucocytes, although marked surface changes were present.

In conclusion, then, sera which in these experiments have increased the phagocytosis of bacteria have concomitantly increased the cohesion, decreased the surface potential difference and altered the wetting properties of the bacteria. Conversely, but with certain exceptions, when sera have altered the surface properties of bacteria as indicated they have caused the bacteria to be spread upon and engulfed by leucocytes. The exceptions have been with aged or heated sera or sera of another species than the leucocytes. These exceptions show the requirements for spreading of the leucocytes to be more delicate than the physical-chemical reactions we have been able to apply. These results are consistent with the conclusion that the various surface reactions including phagocytosis are all dependent upon deposition on the bacterial surface of serum components.

*The Henry Phipps Institute and
the Department of Pathology,
University of Pennsylvania.*

THE ROLE OF HEMOGLOBIN IN THE BLOOD

By A. BAIRD HASTINGS

The purpose of this paper is to present before this symposium the results of certain studies made upon the properties of hemoglobin in various laboratories, with particular attention to those with which the author has been personally associated. Some of these results have already been published; others have not previously been presented. Although these properties will be discussed principally from the standpoint of the influence which they have on the efficiency of the blood as a carrier of oxygen and carbon dioxide, attention will also be paid to the colloidal properties of hemoglobin.

Hemoglobin belongs to the class of crystallizable proteins, but its molecular dimensions and the physical characteristics of its solutions place it among the colloids. From osmotic pressure measurements of hemoglobin solutions containing salts Hüfner¹ concluded that the molecular weight was in the neighborhood of 16,700. This corresponds to the weight of hemoglobin associated with one atom of iron and to the amount of hemoglobin capable of combining with one molecule of oxygen or of carbon monoxide. The more accurate osmotic pressure determinations made by Adair,² in which account was taken of the Donnan Equilibrium, have recently led to the value 66,800, or four times the minimum molecular weight estimated by Hüfner. Confirmation of this value was reported last year by Svedberg and Nichols³ who calculated the molecular weight of carbon monoxide hemoglobin by means of its specific sedimentation velocity and diffusion constant as determined in an oil-turbine ultracentrifuge and found it to be in the neighborhood of 68,000 over the pH range 6.0 to 9.05. The volume of the hydrated particle was calculated to be 110×10^{-21} cc., the volume of water 27.1×10^{-21} cc., the free volume of one molecule 2.99×10^{-23} cc., and the number of water molecules held by a hemoglobin molecule 907. They calculated the surface of a hemoglobin molecule to be 91.95×10^{-14} sq. cm. Taking the cross section of a water molecule (considered a cube) as being 9.64×10^{-16} sq. cm. gave 954 as the minimum number

¹ Hüfner, G., and Gausser, E., *Arch. Physiol.*, 209 (1907).

² Adair, G., *Proc. Roy. Soc. (London)*, 109A, 292 (1925).

³ Svedberg, T., and Nichols, J. B., *J. Am. Chem. Soc.*, 49, 2920 (1927).

of water molecules which would cover the surface of a hemoglobin molecule. The diameter of a hemoglobin molecule calculated from these data is 5.4×10^{-8} mm. or 5μ .

Placed in an electrical field, the particles of hemoglobin move toward the anode at hydrogen-ion concentrations less than 1.8×10^{-7} and toward the cathode in more acid solutions, indicating an isoelectric point ⁴ at pH 6.75.

An exhaustive crystallographic study of hemoglobin prepared from the blood of many species was made by Reichert and Brown.⁵ This monumental work demonstrated the individuality of the hemoglobin of different species. Since this work was done before the importance of the reaction and salt concentration was recognized, the crystals were not formed from isoelectric salt free solutions of hemoglobin. It would be of considerable importance if a crystallographic study comparable to that of Reichert and Brown were made today.

The solubility of oxyhemoglobin prepared by Heidelberger's ⁶ method from the blood of different species has been studied by Landsteiner and Heidelberger.⁷ Their figures are perhaps too high but amply demonstrate the individual solubility of the oxyhemoglobin from different species. For example, horse oxyhemoglobin had a solubility of 12.4 grams per liter and dog oxyhemoglobin 23.8 grams per liter. This illustrates by another property the species specificity of hemoglobin.

A careful study of the solubility of oxyhemoglobin in salt solutions of varying ionic strength has been made by Cohn and Prentiss ⁸ (Fig. 1). They found that the equation

$$\log \frac{S}{S_0} = \frac{2\sqrt{\mu}}{1 + 1.5\sqrt{\mu}}$$

expressed accurately the increase in solubility with increasing ionic strength up to $\mu = 1.0$. S_0 , the solubility in water, exhibited its lowest solubility in solutions of pH 6.6. Recently Cohn and his collaborators have extended their experiments to the solubility of oxyhemoglobin in strong salt solutions where the salting-out effect becomes predominant.

The problem of the state of hemoglobin within the red blood corpuscles is an unsolved one. Its concentration in the cells is approximately 320 grams per kilogram of cells, which far exceeds the solubility of isoelectric oxyhemoglobin in a salt solution of similar ionic strength. The hemoglobin is, however, largely in the form of its potassium salt

⁴ Michaelis, L., and Davidsohn, H., *Biochem. Z.*, 41, 102 (1912).

⁵ Reichert, E. T., and Brown, A. P., *Carnegie Inst. Washington Pub.* 116 (1909).

⁶ Heidelberger, M., *J. Biol. Chem.*, 53, 31 (1922).

⁷ Landsteiner, K., and Heidelberger, M., *J. Gen. Physiol.*, 6, 131 (1923).

⁸ Cohn, E. J., and Prentiss, A. M., *J. Gen. Physiol.*, 8, 619 (1927).

which is highly soluble. There are in addition probably other factors, notably the lipid content of cells, which markedly affect its physical

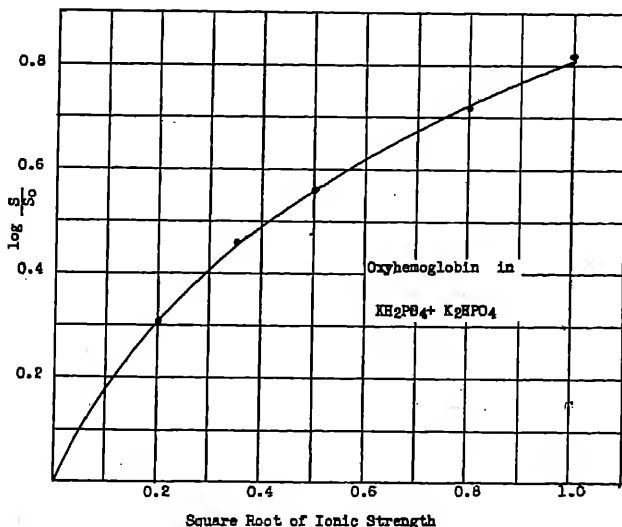


FIG. 1.—The increase in solubility of oxyhemoglobin with increasing ionic strength. From Cohn and Prentiss.

state. This is a problem now being studied by Dr. Meyer Bodansky at the University of Texas.

THE OXYGEN-CARRYING FUNCTION OF HEMOGLOBIN

For the purposes of the present discussion it is sufficient to state that the behavior of hemoglobin in the cells does not seem to be significantly different from its behavior in more dilute solutions.

Boyle⁹ first demonstrated the presence of gases in the blood in 1636. Mayow¹⁰ in 1674 considered the gas pumped off to be nitro-aerial gas or oxygen. Priestley¹¹ in 1776 noticed that blood placed in an atmosphere of hydrogen or nitrogen gave off oxygen. In 1799, Sir Humphry Davy¹² found that 100 parts of blood, when heated, gave off 9 parts of carbon dioxide and 6 parts of oxygen. In spite of these results there was much dispute as to whether or not blood contained any gas at all.

⁹ Boyle, R., "Nova experimenta pneumatica respirationem spectantia," Geneva (1636).

¹⁰ Mayow, J., "Tractatus quinque," Oxonii (1674).

¹¹ Priestley, J., *Phil. Trans., Royal Soc. London*, 66, 226 (1776).

¹² Davy, H., *Ann. Physk. u. Chem.*, 12, 574 (1803).

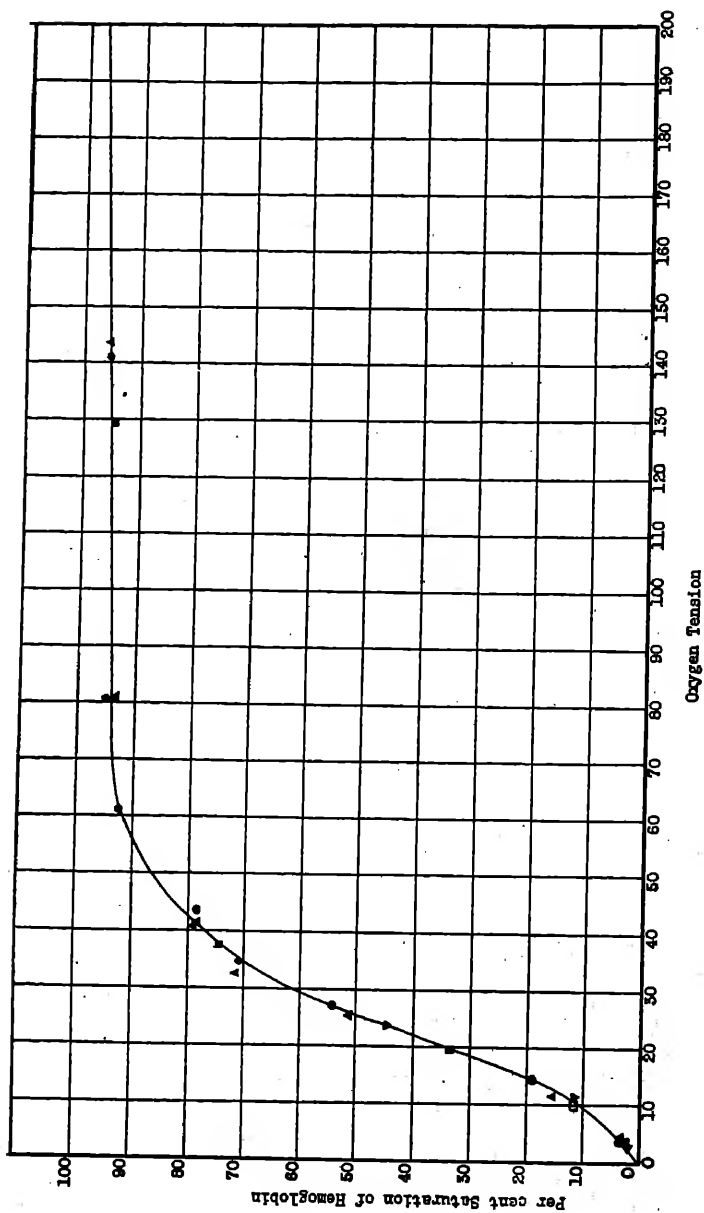


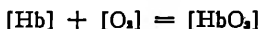
FIG. 2.—Oxygen dissociation curve of horse blood determined at constant pH 7.37.

Magnus¹³ in 1837 introduced more exact methods and by the use of a Torricellian vacuum concluded that blood contained 4 to 8 per cent by volume of carbon dioxide, 1 to 3.5 per cent oxygen and 0.5 to 2.0 per cent nitrogen; further, that arterial blood contained more oxygen than did venous blood.

Following this work came the introduction of various methods of blood-gas analysis which have been undergoing refinement down to the present day. Those most in use are the manometric methods of Haldane,¹⁴ Barcroft¹⁵ and Van Slyke.¹⁶ As the result of the refinement in analytical technique, properties of hemoglobin have come to light which reveal it to be a compound of remarkable fitness for the role which it plays in the blood.

First to be studied quantitatively was the combination of oxygen and hemoglobin. Hoppe-Seyler¹⁷ in 1862 first crystallized and named hemoglobin and proved that it was this constituent of the red blood corpuscles which combined with oxygen but yielded it again in a vacuum. Due to early inaccuracies of technique and partially oxidized preparations of hemoglobin it was thought by Bohr¹⁸ that there were at least four different forms of hemoglobin, each of which was capable of combining with a different amount of oxygen. Later work has definitely established that for every atom of iron in the hemoglobin molecule, there is an oxygen combining capacity of one molecule. This relation persists throughout all the species of hemoglobin studied.

If, for convenience, one designates an iron-bearing equivalent of the hemoglobin molecule by Hb , one may write



Many laboratories, notably those of Haldane and Barcroft in England and of Henderson and Van Slyke in America, have studied this reaction. Briefly, one may summarize the results of these investigators as follows. If in whole blood one determines the percentage of hemoglobin in the form of oxyhemoglobin at varying oxygen tensions and plots the results, one obtains an S-shaped curve which according to A. V. Hill¹⁹ is satisfied by the equation

$$\frac{[\text{Hb}]_n \times [\text{O}_2]^n}{[\text{HbO}_2]_n} = \frac{1}{K}$$

where n has the value 2.2 and K the value of 4.0×10^{-4} at normal blood

¹³ Magnus, *Ann. Phys. u. Chem.*, 40, 583 (1837).

¹⁴ Haldane, J. S., *J. Physiol.*, 22, 465 (1898).

¹⁵ Barcroft, J., and Roberts, F., *J. Physiol.*, 39, 429 (1910).

¹⁶ Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 61, 523 (1924).

¹⁷ Hoppe-Seyler, F., *Virchow's Archiv*, 29, 233 (1864).

¹⁸ Bohr, C., *Skand. Arch. Physiol.*, 3, 47 (1892).

¹⁹ Hill, A. V., *J. Physiol. (Proc.)*, 40, iv (1910).

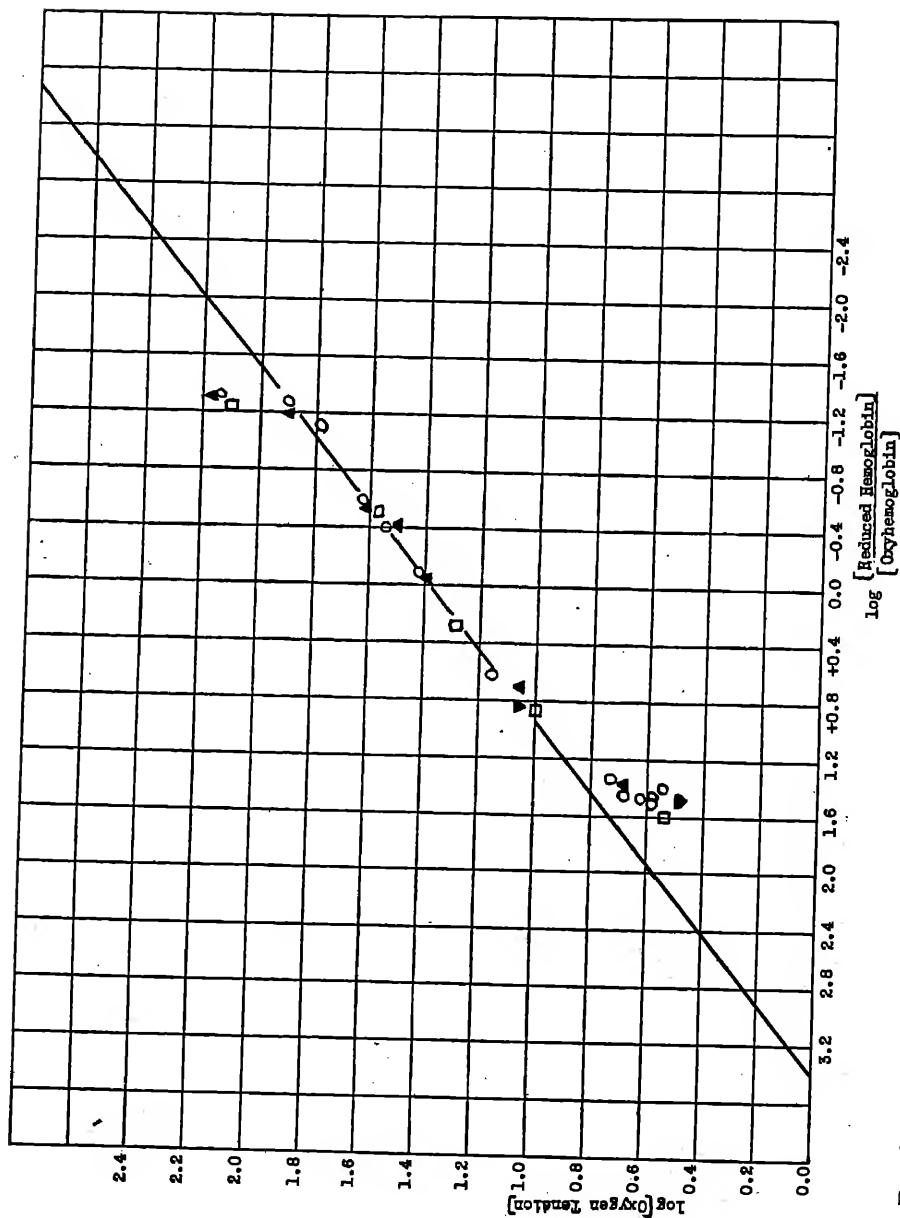


FIG. 3.—Data from Figure 2 plotted as the log (oxygen tension) against the $\log \frac{(\text{Reduced Hemoglobin})}{(\text{Oxyhemoglobin})}$. The straight line is satisfied by the equation $\log \frac{(\text{Reduced Hemoglobin})}{(\text{Oxyhemoglobin})} = -2.43 \log (\text{oxygen tension}) + 2.4$

reaction. Later experiments by Van Slyke, Robson and Hastings,²⁰ in which particular attention was paid to the region below 10 mm. and above 60 mm. oxygen pressure, have demonstrated that the above equation is inadequate to account for the reaction between hemoglobin and oxygen. It was found that n is not a single constant (Figs. 2, 3).

Although an equation may be written which does fit the experimental curve, it is felt that no physical significance can be attached to the constants involved for reasons which will appear when the acidic properties of hemoglobin are considered.

Adair²¹ has recently carefully considered the theories which have been presented to account for the reaction between hemoglobin and oxygen. Finding them all inadequate in some respect, he advanced the following hypothesis. Starting with the fact that hemoglobin has a molecular weight of 66,800 instead of 16,700, he concluded that oxyhemoglobin, designated as $Hb_4(O_2)_4$ is built up and broken down in stages. This hypothesis leads to an equation with so many arbitrary constants that it is impossible to test its correctness by experimental data. It may be stated then that at the present time no completely satisfactory theoretical treatment of the reaction between hemoglobin and oxygen has been presented.

A word should be inserted here regarding the reasons why an hypothesis of adsorption of oxygen by hemoglobin is hardly admissible:

(1) There is distinct chemical individuality between reduced hemoglobin and oxyhemoglobin as measured spectrophotometrically.

(2) They have markedly different solubilities.

(3) They have different isoelectric points, that of oxyhemoglobin being at pH 6.6 and that of reduced hemoglobin being at pH 6.81.

(4) There is a constant ratio of iron content to the oxygen or carbon monoxide combining capacity, namely one atom of iron to one molecule of gas. This, as Stadie and Martin²² have pointed out, provides the most potent argument against the combination being one of adsorption. There is furthermore the additional fact that this relation between iron and oxygen or carbon monoxide holds in hemoglobin from different species which by other criteria are known to be different chemical individuals.

The influence of the reaction and the salt concentration has often been the subject of investigation. There is agreement on the fact that increasing the acidity of the solution causes a decrease in Hill's reaction constant. For horse hemoglobin Van Slyke, Robson and Hastings found

²⁰ Unpublished results.

²¹ Adair, G. S., *J. Biol. Chem.*, 63, 529 (1925).

²² Stadie, W. C., and Martin, K. A., *J. Biol. Chem.*, 60, 191 (1924).

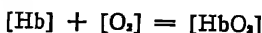
that over the physiological range of pH, this relationship was accurately expressed by the equation

$$\frac{\Delta(-\log K)}{\Delta \text{pH}} = -1.63$$

On the relation between the salt concentration and the reaction between hemoglobin and oxygen, there is not complete agreement. It was originally thought by Barcroft that n was unity in solutions of salt-free hemoglobin and became approximately *two* in solutions of physiological salt concentration. This has subsequently been shown to be incorrect but the exact relation remains for future work to determine.

The physiological significance of the oxygen dissociation curve of hemoglobin is illustrated by the following facts.

In order that the human organism can carry out its normal resting activities it is necessary that approximately 300 cc. of oxygen be distributed to the tissues each minute. To do this with water would require the circulation of 60 liters per minute whereas it is done, with a larger reserve supply of oxygen, by the circulation of 4 liters of blood per minute. Normal blood has an oxygen-carrying capacity of 20 cc. per 100 cc. of blood. Arterial blood, which is in equilibrium with oxygen at a pressure of approximately 100 mm., is 95 per cent saturated and consequently contains 19 cc. of oxygen per 100 cc. of blood; mixed venous blood is about 60 per cent saturated and contains 12 cc. of oxygen per 100 cc. of blood. This corresponds to an oxygen pressure of 30 mm. When for any reason there is required an increased supply of oxygen to the tissues it is readily obtained by a very small decrease in oxygen tension. This is apparent by reference to the oxygen dissociation curve (Fig. 2). A decrease from 40 to 30 mm. in oxygen pressure would cause a decrease in the oxygen content of the blood from 12 to 6 cc. The organism would not be able to draw from the blood an adequate supply of oxygen until the oxygen tension fell to an extremely low level if the reaction between hemoglobin and oxygen were the simple one predicted by the equation



THE CARBON DIOXIDE-CARRYING FUNCTION OF HEMOGLOBIN

The blood must remove from the tissues about 350 cc. of carbon dioxide per minute. To do this with water would require about 35 liters per minute whereas a blood flow of only 4 liters per minute suffices to remove this carbonic acid with a change of only 0.03 in pH. This is made possible by the unique properties of hemoglobin acting as an acid.

Although it is accepted that no significant amount of carbon dioxide

is combined with hemoglobin under normal physiological conditions, hemoglobin is nevertheless responsible in large measure for the ability of the blood to take up carbon dioxide from the tissues and give it up in the lungs without a marked change in the reaction of the blood. This property of the blood is the result, first of the high buffer value of the hemoglobin molecule and second, of the increase in the acidity of hemoglobin upon oxygenation. Van Slyke²³ in 1922 calculated the buffer value of blood, $\Delta\text{Ba}/\Delta\text{pH}$, to be 0.0221 and predicted that this was largely due to the weak acid groups of hemoglobin. This figure was found to be essentially correct, 75 per cent of the buffering being due to the hemoglobin present. In terms of what this means to the organism, one may state that a buffer value of this magnitude permits the blood to take up from the tissues 0.66 millimols of acid per liter with a change of 0.03 in pH. But one liter of venous blood contains about 2.4 millimols more acid than does arterial blood and yet the pH change is only 0.03.

Put differently, the pH difference between arterial and venous blood would be 0.12 pH instead of 0.03 were it not for the property of hemoglobin which will now be described.

The influence of oxygen upon the carbon dioxide carried by blood was first demonstrated by the now classic experiments of Christiansen, Douglas and Haldane²⁴ who showed that the amount of carbon dioxide carried by venous blood was greater than by arterial blood at the same carbon dioxide tension. L. J. Henderson²⁵ suggested that this phenomenon might be due to the oxyhemoglobin being a stronger acid than reduced hemoglobin. Using Haldane's data, Henderson made the first calculation of the degree to which the acidity of hemoglobin would need to be increased by oxygenation. His figure of $K_o/K_R = 9$ was acknowledged to be only a first approximation.

A study of the acid properties of hemoglobin was made by Hastings, Van Slyke, Neill, Heidelberger and Harington.²⁶ Crystals of isoelectric salt-free oxyhemoglobin were dissolved in a known amount of base. These solutions were equilibrated in a water bath at varying carbon dioxide tensions and analyzed for their total and oxyhemoglobin content, their carbon dioxide content, and the carbon dioxide tension. The pH was calculated from the carbon dioxide tension and content using the mass law equation. The dissociation constant of carbonic acid in hemoglobin solutions was determined in a separate series of experiments. From the total alkali added and the bicarbonate content of the solution, the base minus the acid bound by the hemoglobin

²³ Van Slyke, D. D., *J. Biol. Chem.*, 52, 525 (1922).

²⁴ Christiansen, C., Douglas, C. G., and Haldane, J. S., *J. Physiol.*, 48, 244 (1914).

²⁵ Henderson, L. J., *J. Biol. Chem.*, 41, 401 (1920).

²⁶ Hastings, A. B., Van Slyke, D. D., Neill, J. M., Heidelberger, M., and Harington, C. R., *J. Biol. Chem.*, 60, 89 (1924).

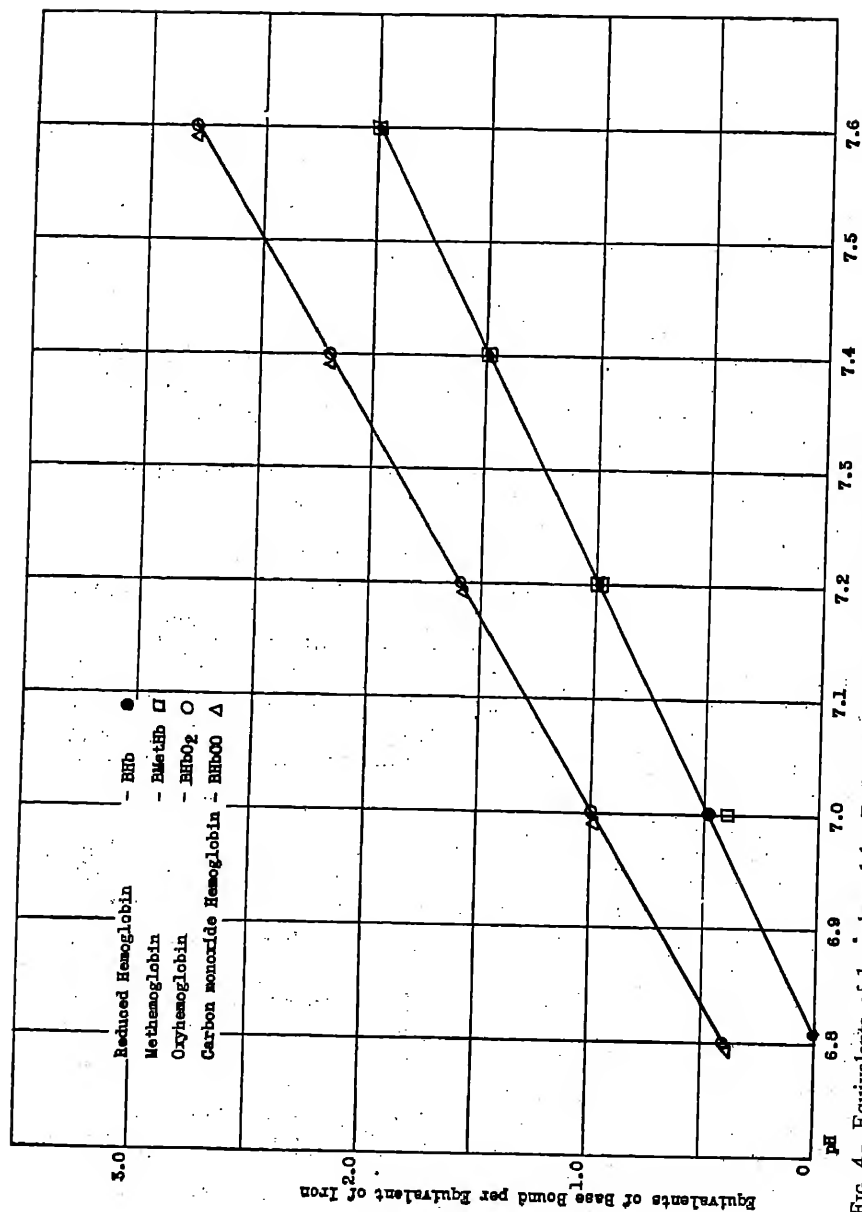


Fig. 4.—Equivalents of base bound by Reduced Hemoglobin, Methemoglobin, Oxyhemoglobin and Carbon Monoxide Hemoglobin plotted against pH.

was calculated. The data were then plotted as the base minus the acid bound by hemoglobin against the pH, for reduced and oxygenated hemoglobin, carbon monoxide hemoglobin and methemoglobin. The consistency of the data is shown in Table I and Figure 4, which gives the base minus acid bound by different preparations of hemoglobin in the forms mentioned. This figure in addition illustrates the following facts: (1) that reduced hemoglobin and methemoglobin have, over the physiological pH range, the same capacity for combining with base; (2) that carbon monoxide and oxyhemoglobin have the same capacity to combine with base; and (3) that oxyhemoglobin combines with more base than does reduced hemoglobin.

In order to test Henderson's hypothesis it was necessary to study the effect of oxygen on the acidity of hemoglobin over as wide a range of reaction as possible. One would expect a change in the acidity of a carboxyl group to be evident over a range of two pH units. By the method just outlined a range of only one pH unit was possible. An extension of the range tested was affected in the following manner. By the carbon-dioxide-saturation method it was demonstrated that over the physiological pH range, oxygen and carbon monoxide had the same effect upon the base bound by hemoglobin. It was further demonstrated that 10 mm. of carbon monoxide, sufficient to keep hemoglobin in the form HbCO , did not interfere with hydrogen electrode measurements. Electrometric titrations of reduced hemoglobin and carbon monoxide hemoglobin were then made from pH 6.2 to 8.6. The effect of carbon monoxide on the titration curve was in accord with that predicted by Henderson's hypothesis.²⁷

TABLE I. *Equivalents of Base Bound by Different Forms of Hemoglobin, Per Iron Equivalent, at pH 7.40.*

Horse Hemoglobin — Total Base = 30 mM per liter.

	$\frac{\text{BHb}_R}{\text{Hb}_R}$	$\frac{\text{BMetHb}}{\text{MetHb}}$	$\frac{\text{BHbO}_2}{\text{HbO}_2}$	$\frac{\text{BHbCO}}{\text{HbCO}}$
1.....	1.46	1.50	2.15	2.18
2.....	1.57	1.54	2.24	2.18
3.....	1.56	1.61	2.22	2.22
4.....	1.47	1.46	2.10	2.19
5.....	1.32	...	2.02	2.18
6.....	1.54	...	2.20	...
Average.....	1.54	1.53	2.18	2.19

From these data empirical equations relating the base bound by hemoglobin, the pH, and the degree of oxygenation of the hemoglobin were derived. For horse hemoglobin the equation is

²⁷ Hastings, A. B., Murray, C. D., Sendroy, J., and Heidelberger, M., *J. Biol. Chem.*, **61**, 317 (1924).

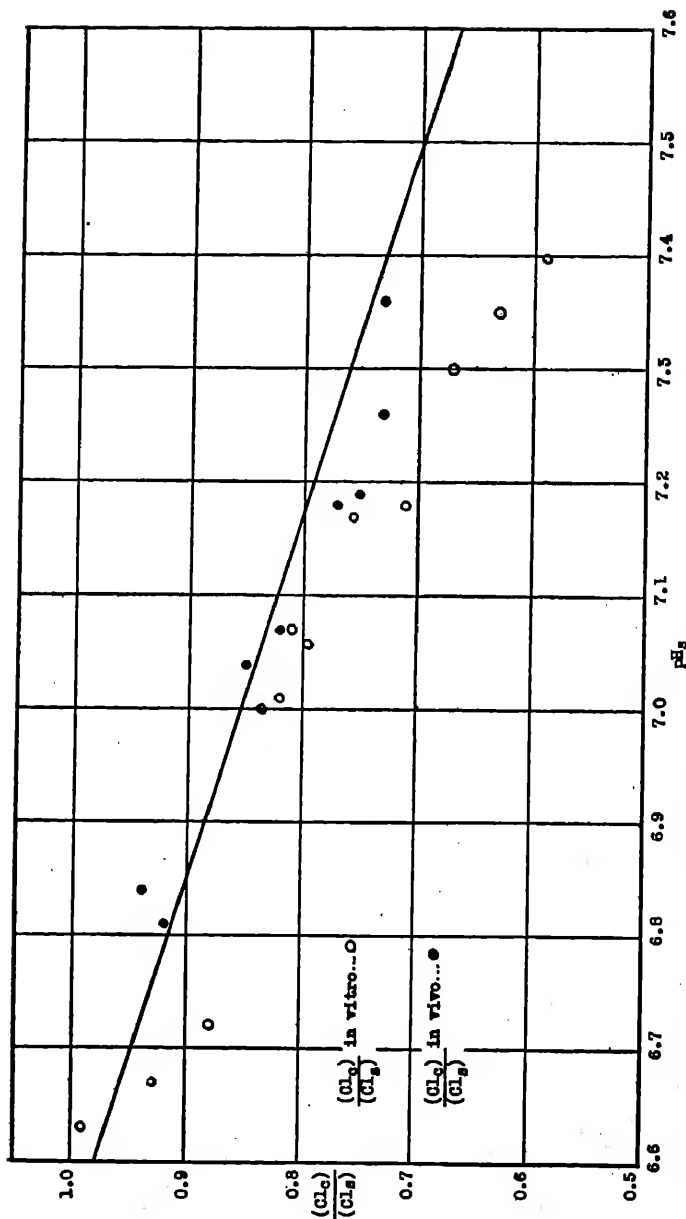


FIG. 5.—The distribution of chlorides between serum and ce'lls in dog blood, plotted against the pH of serum. The open circles, ○, denote the distribution in blood whose pHs were changed in vitro. The solid circles, ●, the distribution in experimental acidosis. The solid line is the ratio predicted by the Van Slyke, Wu and McLean equation.

$$\text{BHb} = 2.6(\text{Hb})(\text{pH} - 6.81) + (\text{HbO}_2) \left(\frac{1}{1 + 10^{6.87 - \text{pH}}} - \frac{1}{1 + 10^{8.33 - \text{pH}}} \right)$$

For dog hemoglobin it is

$$\text{BHb} = 2.0(\text{Hb})(\text{pH} - 6.81) + (\text{HbO}_2) \left(\frac{1}{1 + 10^{6.98 - \text{pH}}} - \frac{1}{1 + 10^{8.22 - \text{pH}}} \right)$$

These equations demonstrate species differences in the composition of hemoglobin which markedly affect their titration curves and indirectly influence the carbon dioxide-carrying function of the blood.

THE EFFECT OF HEMOGLOBIN ON THE DISTRIBUTION OF DIFFUSIBLE IONS IN THE BLOOD

One may now consider the blood as a whole and in what manner the presence of hemoglobin in the red blood corpuscles influences the distribution of such diffusible ions as chloride, bicarbonate and hydrogen. Following the determination of the base bound by hemoglobin at varying pH values and the effect of oxygenation upon its titration curve, this influence was quantitatively evaluated by Van Slyke, Wu, and McLean.²⁸ The unequal distribution of chloride and bicarbonate ions between the serum and cells of blood (Zuntz, 1868)²⁹ and the fact that adding carbon dioxide to the blood caused the migration of chlorides from the serum into the cells had long been recognized (Gürber, 1895),³⁰ but no satisfactory explanation was forthcoming until Van Slyke applied the theory of membrane equilibrium to the problem.

Taking into account the fact that erythrocytes are impermeable to cations and to proteins, and assuming that (1) electrical neutrality exists in the two phases serum and cells, (2) that there is osmotic equilibrium and (3) that the Donnan theory of membrane equilibria predicts the distribution of the diffusible ions, equations relating the diffusible and non-diffusible constituents were deduced. In terms of the non-diffusible factors of the system the distribution ratio

$$r = 1 - \frac{(\text{BP})_c + (\text{Hb})_c - (\text{BP})_s}{2([\text{B}]_s - [\text{BP}]_s)} = \frac{(\alpha\text{H})_s}{(\alpha\text{H})_c} = \frac{(\alpha\text{Cl})_c}{(\alpha\text{Cl})_s} = \frac{(\alpha\text{HCO}_3)_c}{(\alpha\text{HCO}_3)_s}$$

where the symbols have the following meanings:

- (BP)_c = Base bound by hemoglobin.
- (Hb)_c = Molecular concentration of hemoglobin.
- (BP)_s = Base bound by serum proteins.
- (B)_s = Total base concentration in the serum.
- (α)_s = The ionic activity in the serum.
- (α)_c = The ionic activity in the cells.

²⁸ Van Slyke, D. D., Wu, H., and McLean, F. C., *J. Biol. Chem.*, 56, 765 (1923).

²⁹ Zuntz, N., "Beiträge zur Physiologie des Blutes," Bonn (1868).

³⁰ Gürber, A., *(Maly's) Jahresber.*, 25, 164 (1895).

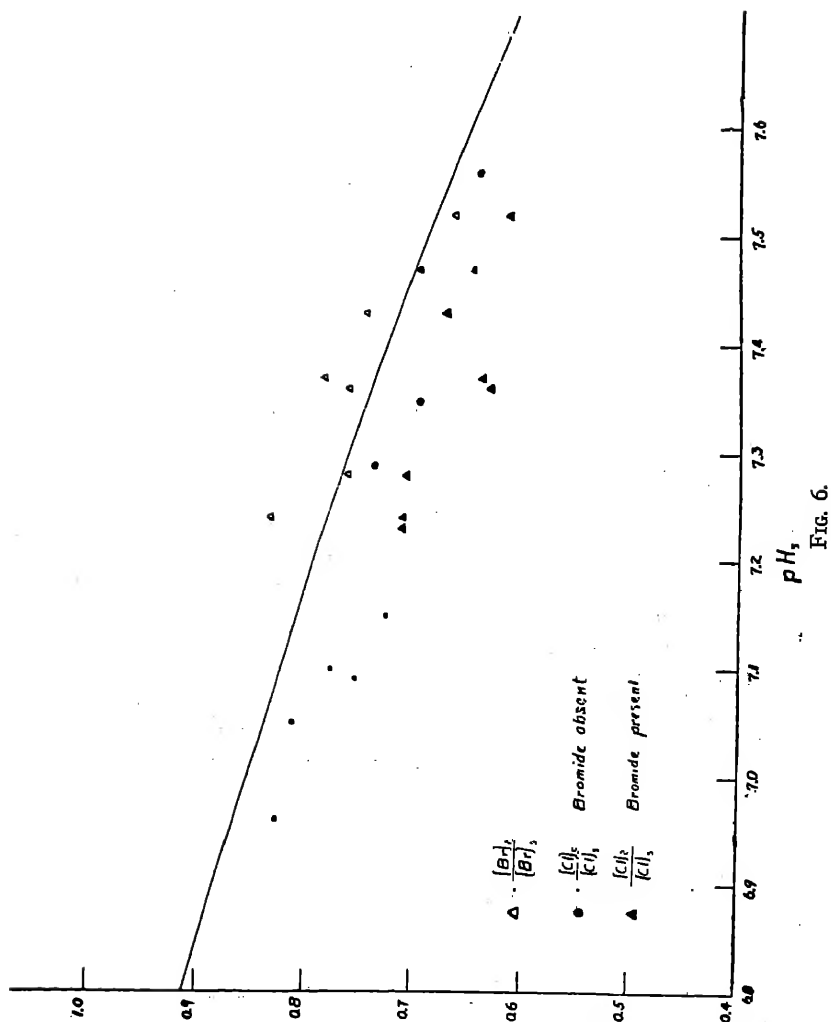


FIG. 6.

Substituting the equation for the base bound by the hemoglobin and the serum proteins, Van Slyke obtained the relationship

$$r = 1 - \frac{3.35(\text{Hb})_c (\text{pH}_c - 6.44) - 0.068 P_s (\text{pH}_c - 4.80) + (\text{O}_2)_c (0.25\text{pH}_c - 1.18)}{2[(\text{B})_s - 0.0068[\text{P}]_s (\text{pH}_c - 4.80)]}$$

It was found that the actual distribution of chloride and bicarbonate was very close to that predicted by their equation and, what is more important, the change in the distribution with change in pH was in accord with the predicted change. A further test of the validity of the hypothesis was made by Van Slyke, Hastings, Murray and Sendroy⁸¹ by determining the effect of oxygenation and reduction upon the chloride-, bicarbonate- and hydrogen-ion distribution. The increased acidity of hemoglobin with oxygenation means increased base bound at constant pH. This in turn means an increased concentration of non-diffusible ions within the red cells and consequently a lowered distribution ratio of the diffusible ions. The decrease in ratio corresponded closely with that predicted by the Van Slyke, Wu and McLean equation.

The author, with H. N. Harkins,⁸² has lately further extended the application of the hypothesis to the prediction of the distribution of bicarbonate and chloride ions in the blood in experimental acidosis, (Fig. 5) and, in conjunction with Dr. Louis Leiter,⁸³ in experimental alkalosis. Furthermore, in studies made with Dr. Van Dyke⁸⁴ upon the distribution of bromide when added to blood it was found that although the change in the bromide ratio with change in pH and degree of oxygenation of the hemoglobin was in accord with that predicted by the Van Slyke, Wu and McLean equation, the absolute value of the ratio was higher than, instead of equal to, the chloride distribution ratio (Fig. 6). This discrepancy became particularly marked when the blood of dogs fed larger amounts of sodium bromide was analyzed. Thus it seems that hemoglobin acting as a non-diffusible constituent of the cells is not the only factor concerned with the distribution of the diffusible ions. Further work on this problem is in progress.

SUMMARY

In conclusion then, one may summarize the physiologically important properties of hemoglobin as being

(1) that of reacting with oxygen in such a manner that, in the environment provided by the organism, it takes up oxygen from the lungs and delivers it to the tissues with maximal efficiency;

⁸¹ Van Slyke, D. D., Hastings, A. B., Murray, C. D., and Sendroy, J., *J. Biol. Chem.*, 65, 701 (1925).

⁸² Unpublished results.

⁸³ Unpublished results.

⁸⁴ Hastings, A. B., and Van Dyke, H. B., *J. Biol. Chem. (Proc.)*, 78, 35 (1928).

(2) that of providing the blood with 90 per cent of its capacity to buffer acids by virtue of its constituent weak acid groups and its unique capacity to increase its acidity upon oxygenation;

(3) that of controlling in large measure the distribution of diffusible ions between the serum and cells.

*University of Chicago,
Chicago, Illinois.*

THE EFFECT OF EMULSIFICATION IN THE PEPTIC SYNTHESIS OF PROTEIN

BY H. WASTENEYS AND H. BORSOOK

INTRODUCTION

When pepsin is added to a concentrated peptic hydrolysate of protein a precipitate is formed which may be proved on analysis to be more complex in composition than the original digest and is, in fact, protein.

This precipitation was first observed by Danilewski in 1886.¹ The methods then available were not adequate to prove conclusively that a synthesis of protein had been effected although Danilewski and subsequent Russian workers from 1896 to 1908,² believed that such synthesis had been accomplished. One of these workers, Sawjalow² gave the name *plastein* to the precipitate.

Protein synthesis by pepsin was studied by T. B. Robertson³ in 1907-8, and in the same years A. E. Taylor⁴ reported a synthesis of protein by trypsin from a tryptic digest of protamine.

In 1911 and 1912 Henriques and Gjaldbæk⁵ provided the first evidence for the assumption that the complexity of the *plastein* molecule is greater than the material from which it has been precipitated. They found that the ratio of free amino to total nitrogen is lower for *plastein* than for any of its precursors. The diminution in this ratio which they observed was, however, small, owing to the fact that their estimations were not made on the isolated *plastein* but on the digest before and after precipitation had occurred.

In 1924 Wasteneys and Borsook investigated the possibility of synthesizing proteins by concentrating protein digests on the surface of artificial cells formed from chloroform and lecithin emulsions according to the methods of Robertson³ and Newton Harvey.⁷ They were unsuccessful in their attempt owing to the fact that they worked with trypsin on tryptic digests, which subsequent experience has shown to be re-

¹ Danilewski quoted from Henriques, and Gjaldbæk, *Z. Physiol. Chem.*, 71, 485 (1911).

² Sawjalow, *Arch. ges. Physiol.*, 85, 171 (1901); Kurayeff, *Hofmeister's Beitr.*, 1, 121 (1901); 2, 411 (1902); Lawrow and Salaskin, *Z. Physiol. Chem.*, 36, 277 (1902); Sawjalow, *ibid.*, 54, 119 (1907-08).

³ Robertson, *J. Biol. Chem.*, 3, 95 (1907); 5, 493 (1908-09).

⁴ Taylor, *J. Biol. Chem.*, 3, 87 (1907); 5, 381 (1908-09).

⁵ Henriques and Gjaldbæk, *Z. Physiol. Chem.*, 71, 485 (1911); 81, 439 (1912).

⁶ Robertson, *J. Biol. Chem.*, 4, 1 (1908).

⁷ Harvey, *Biochem. Bull.*, 2, 50 (1912); *Science (N.S.)*, 36, 564 (1912).

fractory material for this purpose. Working with pepsin on concentrated peptic digests, the aid of emulsions was found superfluous and a 40 per cent yield of synthesized product could be readily obtained. Wasteneys and Borsook⁸ proved the synthetic product to be a protein possessing all the characteristics which distinguish a protein from its cleavage products. The highest yields of synthesized protein are obtained with a moderately concentrated digest, that is, one containing the nitrogen equivalent of a 30 per cent solution of the original protein.⁹ The optimum pH for synthesis is 4.0 and the enzyme concentration need not exceed 2 per cent.¹⁰ Higher rates of synthesis are obtained with increasing temperature up to 72° C., the point at which the enzyme, even in the presence of concentrated substrate, is almost immediately destroyed.¹¹ The synthesizability of a peptic digest is further found to be inversely proportional to the length of time it has been exposed to the hydrolytic action of the enzyme.

The ease with which protein synthesis from concentrated digests is obtained by the unaided enzyme had diverted the attention of the authors from the possible use of emulsions as an auxiliary to the enzyme. In 1926 their attention was recalled to this possibility by T. B. Robertson¹² who summarized his ideas in this connection as follows:

"Following the suggestion of H. R. Marston, that the surfaces of the mitochondria are the loci of syntheses in the cell, it is pointed out that the lipid composition of these bodies would tend to orient molecules at their surfaces in such a manner that the reactive groups, for example, the carboxyl and alpha-amino groups of amino acids, would all point towards the aqueous phase, while the lyotrope hydrocarbon chains would be buried in the lipid phase, the movements of the molecules would thus be restrained and confined to rotation around the axis of the hydrocarbon chain, so that the reactive groups would attain an effective concentration, at the surface of the aqueous phase, which could not possibly be attained in any other manner. It is suggested that this affords an explanation of the completeness of enzymatic syntheses in living tissues, which has not hitherto been attainable *in vitro* by mere concentration of the hydrolysate through removal of water from the reacting system."

Marston¹³ observed an acceleration of the rate of synthesis by trypsin when the lipoids of egg-yolk were added with the enzyme.

The results to be described represent an attempt to investigate the influence, if any, of the emulsification of the substrate during peptic

⁸ Wasteneys and Borsook, *J. Biol. Chem.*, 62, 15 (1924).

⁹ Borsook and Wasteneys, *J. Biol. Chem.*, 53, 563 (1925).

¹⁰ Wasteneys and Borsook, *J. Biol. Chem.*, 62, 675 (1925).

¹¹ Borsook and Wasteneys, *J. Biol. Chem.*, 62, 633 (1925).

¹² Robertson, *Australian J. Exp. Biol. Med. Sci.*, 3, 97 (1926).

¹³ Marston, *Australian J. Exp. Biol. Med. Sci.*, 3, 233 (1926).

synthesis. They fail to confirm Robertson's prediction in all details, for fatty emulsions were found to be ineffective. When, however, emulsions formed with benzene or its derivatives are present with the digest and enzyme in appropriate amount, a marked acceleration of the rate of synthesis is induced. Among all the emulsifying materials tried, benzene and benzaldehyde have proven to be most effective. Chloroform is slightly efficient, xylol, talc, kieselguhr and barium sulfate are less efficient, and fats and fatty acids are ineffective.

When a digest is employed which has been exposed to the action of the enzyme for the shortest time necessary for the complete disappearance of the protein, there is no augmentation by benzaldehyde of the yield of synthesized protein at equilibrium. And indeed it has been found impossible, so far, to effect any increase in the yield over that obtained by using the unaided enzyme under optimal conditions for synthesis by pepsin alone.

It should be mentioned, however, that in digests where the preparatory hydrolysis has been prolonged beyond the time when protein has disappeared and the yield of synthesized protein on treatment of the concentrate by pepsin has been consequently reduced below the optimum obtainable from an early digest, the efficient emulsifying agents then cause a considerable augmentation of the equilibrium yield. Nevertheless, even though there is no augmentation of the yield at equilibrium there is under all conditions with benzene, benzene derivatives, and chloroform, a marked acceleration of the initial rate of synthesis.

Analysis of the control experiments suggested that synthesis can be effected without enzyme by the emulsifying agent alone if it is an accelerator of the enzymatic synthesis. Such syntheses were repeated many times. The yield with emulsifying agents alone was sufficiently large in the cases of benzene and benzaldehyde to permit isolation and characterization of the proteins so synthesized.

These effects of emulsifying agents on the peptic synthesis of protein are probably examples of specific adsorption. The valencies involved, it seems, are secondary rather than primary in Langmuir's interpretation of these terms.¹⁴

This is suggested by the efficiency of such inert chemical compounds as benzene, toluol, and chloroform. The emulsifying agent, while incompletely removed by repeated washing with water, is entirely separated by alcohol, leaving a protein which contains the same percentage of nitrogen as in that synthesized by enzyme alone.

The chemical and physical properties of the proteins synthesized varied with the means employed in their synthesis. On comparing the titration curves of proteins synthesized by pepsin alone, by pepsin +

¹⁴ Langmuir, *J. Am. Chem. Soc.*, 39, 1848 (1917).

benzaldehyde, by benzaldehyde alone and by benzene it was found that the protein synthesized by benzene alone possessed the highest base combining capacity. That synthesized by benzaldehyde alone had a similar but lower combining capacity while those proteins synthesized by pepsin alone and by pepsin + benzaldehyde combined with much less base.

The ratios of free amino nitrogen and free carboxyl groups to total nitrogen are different in the proteins synthesized by the above methods and there are distinct differences in hydrolysability by pepsin.

The solubilities also show marked differences. For example, on the alkaline side of neutrality the proteins prepared by pepsin, either with or without benzaldehyde, are soluble only at alkalinities greater than about pH 7.7. At about pH 7.6 these proteins flock out of solution almost completely but the proteins prepared in the absence of enzyme with either benzene or benzaldehyde are quite soluble at neutrality and are not precipitated until the benzaldehyde protein reaches pH 6.0 and the benzene protein reaches pH 5.0.

These results suggest that proteins of different chemical composition have been obtained, presumably through variations in the orientation of the participating components of the digest effected in a specific manner by the particular emulsifying agent employed. Viewed in this light the enzyme itself may be considered merely as an emulsifying agent, yielding a protein peculiar to its orienting effects and effecting the synthesis at a rate different, and in the nature of the case greater than, other emulsifying agents. This variation in physical and chemical properties with the emulsifying agent employed suggests a possible mechanism by which the many proteins of the organism may be synthesized, as they are, *in vivo*, from a common substrate.

EXPERIMENTAL PROCEDURE

Pepsin, Merck, and Egg Albumin, Merck, were used throughout the experiments. The Albumin was digested with 0.2 per cent pepsin at pH 1.6 at 37° C. for lengths of time which varied promiscuously. In every case, however, the digestion was continued at least until the hydrolysate gave no precipitate with trichloroacetic acid. This variation in time of digestion was subsequently found to be responsible for varying equilibrium yields of protein when the product was used in synthesis experiments.

To prepare the digest for synthesis, the reaction was adjusted to pH 4.0 with concentrated alkali, it was then heated for an hour on a boiling water bath and filtered. The perfectly clear filtrate was concentrated until the concentration was equivalent to about 25 per cent in terms of

original protein. During the process of concentration the reaction remained unchanged.

THE EFFECT OF EMULSIFICATION ON THE RATE OF SYNTHESIS

10 cc. of the digest, 1 cc. of aqueous 10 per cent pepsin and 1 cc. of the emulsifying agent (or approximately 1 gram of powder emulsifier) were mixed in a suitable number of test tubes, shaken vigorously for 1 minute, stoppered, and placed in a water thermostat at 37° C. for the times indicated. When desired, two of the tubes (duplicates) were removed and diluted 10 times with distilled water for greater ease in treatment and because the protein is soluble in concentrated digest. The protein was estimated in one of two ways, both of which yielded similar results since in dilute solutions at pH 4.0 the protein is insoluble in all cases. The whole suspension was filtered and the protein on the filters was washed until chlorine free, and all soluble nitrogenous constituents had been removed.

In one method the amount of protein present was determined by a Kjeldahl determination on the whole precipitate and the filter paper. In the other method trichloroacetic acid was added to an aliquot of the diluted digest to a concentration of 2 per cent. The amount of protein synthesized was determined by the Kjeldahl method and was the difference between the protein equivalent of nitrogen before and after filtration. The results of these experiments are given in Table I which shows also the results of control experiments where the emulsifying agent was added as before but inactive pepsin was used in place of active pepsin.

TABLE I. *The Effect of Emulsifying Agents on the Rate of Synthesis.*

Emulsifying Agent	Synthesis in 24 Hours with Active Pepsin	Synthesis in 24 Hours with Inactive Pepsin
	Milligrams protein N in terms of 10 cc. of original digest	Milligrams protein N in terms of 10 cc. of original digest
None	10	nil.
Oleic acid	9	nil.
Olive oil	9	nil.
Barium sulfate	14	nil.
Talc powder	15	nil.
Kieselguhr	14	nil.
Chloroform	19	nil.
Benzene	38	8
Toluene	24	4
Xylol	16	1
Benzoic acid	25	4
Benzaldehyde	51	11

Table I shows, as mentioned above, that fatty substances have no augmenting effect on synthesis. The three powders used, and also xylol, and chloroform, show a definite and similar effect in increasing the yield in 24 hours. Toluene, benzene and benzoic acid show a greater effect. Benzaldehyde has increased the rate of synthesis in this experiment 500 per cent. Even when inactive pepsin was employed, in the controls, benzene and its derivatives showed indications of small but quite definitely positive synthesis. This was the first suggestion that synthesis might be effected by an emulsifying agent alone, in the absence of active enzyme.

In Table II and Figure 1 the rate of synthesis is studied in more detail with both active and inactive pepsin. The preparation of the mixtures and the method of analysis were the same as in the previous experiment.

TABLE II. *The Effect of Benzaldehyde and Chloroform on the Rate of Peptic Synthesis.*

Time Hours	Amount of Synthesis		
	Benzaldehyde + Pepsin Milligrams Protein N.	Chloroform + Pepsin Milligrams Protein N.	Pepsin Alone Milligrams Protein N.
1.....	25	..	4
3.....	44	..	7
6.....	53	..	11
12¼.....	77	..	24
24¼.....	84	64	32
47¼.....	100	..	50
72.....	130	..	63
143¼.....	140	89	65
With inactive in place of active pepsin			
23.....	8	nil.	nil.
143.....	20	nil.	nil.

A comparison of the figures for benzaldehyde + pepsin with those for pepsin alone in Table II shows the typical marked accelerating influence of the benzaldehyde emulsion. The previously described synthesis with emulsifying agents alone, in this case benzaldehyde, is also confirmed.

The slopes of the curves in Figure 1, plotted from the results in Table II, indicate that equilibrium had not been attained in these experiments in 24 hours. Subsequent experience showed that a number of days are required under these conditions before equilibrium is approximated. This is demonstrated by the results in Table III and by Figure 2.

These results show not only the previously observed acceleration of the rate by the benzaldehyde emulsion with pepsin but also an unequivocal augmentation of the equilibrium yield. A synthesis with the benzaldehyde emulsion alone is demonstrated by the values for synthesis steadily

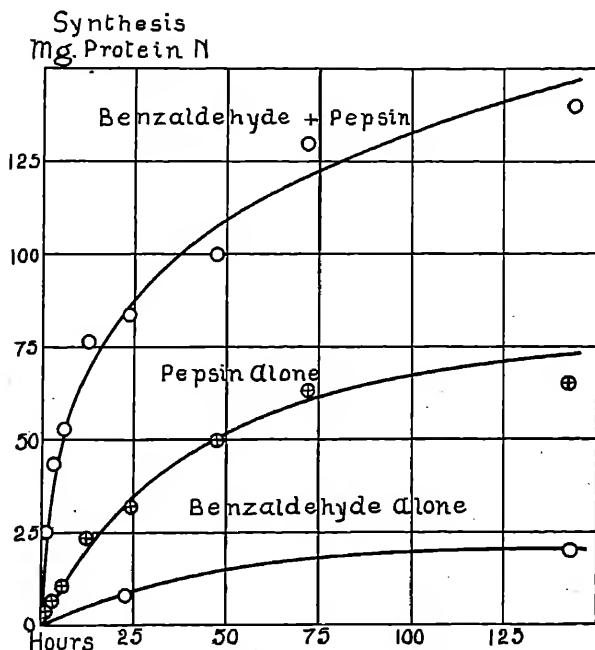


FIG. 1.—The effect of benzaldehyde emulsion on the rate of protein synthesis.

rising with increasing time, even after 400 hours. Where active pepsin was present equilibrium was attained in both cases in approximately 300 hours.

Of all the substances yet tried benzaldehyde is the most active accelerator of the early synthesis. Benzene, however, though not so active in the early stages, eventually augments the equilibrium yield to the same level as that reached with benzaldehyde. This is shown by the results of three groups of experiments with different digests which are set out in Table IV. The digests were all obtained from the same original sample of albumin and pepsin. At the times indicated under duration of digestion, a large aliquot was removed, boiled, and prepared for synthesis in the manner described above. In addition to the augmenting effects of benzene and benzaldehyde the results in Table IV

TABLE III. *The Effect of Benzaldehyde on the Equilibrium in Protein Synthesis.*

Time Hours	Amount of Synthesis			
	Benzaldehyde+ Active Pepsin	Active Pepsin Alone	Benzaldehyde+ Inactive Pepsin	Inactive Pepsin Alone
	Milligrams Protein N.	Milligrams Protein N.	Milligrams Protein N.	Milligrams Protein N.
6.....	37	9	..	nil.
12.....	70	26	..	nil.
27.....	93	34	10	nil.
54.....	100	41	..	nil.
75.....	123	52	..	nil.
77.....	131	58	15	nil.
144.....	136	82	23	nil.
216.....	161	91	21	nil.
288.....	156	95	26	nil.
342.....	175	105	..	nil.
386.....	185	108	34	nil.
414.....	177	103	36	nil.

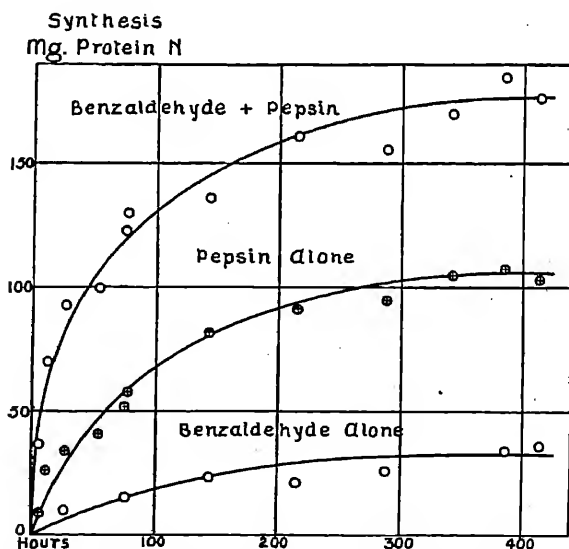


FIG. 2.—The effect of benzaldehyde emulsion on equilibrium in protein synthesis.

show the progressive diminution in the yield, with the duration of the hydrolysis, whether or not emulsifying agent is used with pepsin. The result obtained with the 4 day digest, it seems, is especially significant. Here, where the highest yield is obtained with pepsin alone, benzaldehyde exerted no augmenting effect. It has been shown¹⁵ that a slow secondary

¹⁵ McFarlane, Dunbar, Borsook, and Wasteneys, *J. Gen. Physiol.*, 10, 437 (1927).

hydrolysis of the primary products occurs during prolonged peptic digestion. The results given in Table IV indicate that a factor essential in peptic synthesis disappears during this long period of contact of active enzyme with the dilute solution of proteoses and peptones. It

TABLE IV. *The Effect of Benzaldehyde and Benzene on the Rate and on the Equilibrium Position in Peptic Synthesis.*

Duration of Digestion	Time	Amount of Synthesis		
		Benzaldehyde +	Benzene +	Pepsin
		Active Pepsin	Active Pepsin	
Days	Days	Milligrams Protein N.	Milligrams Protein N.	Milligrams Protein N.
13	5	72	66	54
	10	75	74	54
	15	76	75	54
21	5	36	38	29
	10	43	45	37
	15	45	49	36
46	6	33	33	..
	12	36	42	30
	18	38	43	30
4	18	113	..	112
	24	114	..	112

seems that pepsin on the one hand, and benzene and benzaldehyde on the other, are so rendered progressively less efficient to induce the concentration and orientation of the cleavage products which results in their re-synthesis. The property being so influenced by pepsin is lost more rapidly than the corresponding relation to benzaldehyde or benzene. On the other hand, a digest which has been exposed to the hydrolytic action of pepsin for only a short time, is synthesizable to the same extent by pepsin alone as by the combined action of an emulsifying agent and enzyme.

It was of some interest to determine the influence of the amount of benzaldehyde, with and without active enzyme, on the equilibrium yields of protein synthesized. The details of preparation were the same as in the previous experiments. Varying amounts of benzaldehyde were added as indicated in Table V and Figure 3. The mixtures were incubated at 37° C. for 12 days.

It is interesting to note that the appearance and viscosity of the mixtures used in Table V, which had exhibited a progressive change with increasing amounts of benzaldehyde, showed a sudden change in that mixture where the diminution in the yield of synthesized protein began. It was observed that the opacity, when the mixtures were shaken, increased with increasing amounts of benzaldehyde, up to 5 cc. As

TABLE V. *The Effect of the Amount of Benzaldehyde Used on the Synthesis of Protein with Active and Inactive Enzyme, in 12 Days at 37°C.*

Benzaldehyde Used Cubic Centimeters	Amount of Synthesis	
	With Active Pepsin Milligrams Nitrogen	With Inactive Pepsin Milligrams Nitrogen
0.0.....	87	11
0.25.....	155	28
0.5.....	161	40
0.75.....	175	49
1.0.....	200	56
2.0.....	203	68
3.0.....	208	79
5.0.....	207	92
7.0.....	204	62
8.0.....	209	47
9.0.....	190	49
10.0.....	70	10

the opacity increased, the viscosity diminished. In the mixtures containing larger amounts of benzaldehyde than 5 cc. the emulsion was no longer creamy white in color but brown, much less opaque, and markedly less viscous.

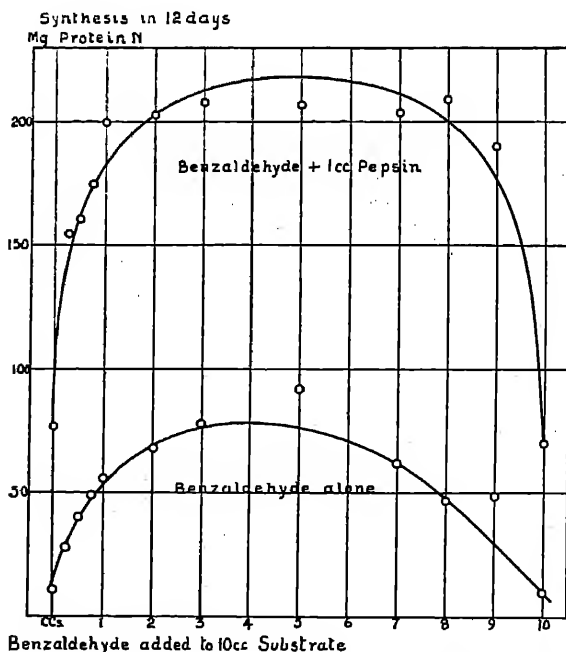


FIG. 3.—The effect of the amount of benzaldehyde used in emulsification on the quantity of protein synthesized in 12 days.

These changes suggest that when as much as 5 cc. of benzaldehyde are added to 10 cc. of digest and 1 cc. of pepsin solution, the form of the emulsion, as compared with its form in the presence of lesser amounts of benzaldehyde, changes suddenly, probably from a benzaldehyde in water to a water in benzaldehyde emulsion. With this inversion, changes necessarily occurring in the concentration and orientation of the cleavage products, result in a reduction of the yield of protein synthesized.

In the course of the experiments described above the proteins prepared by the various agents appeared to vary in their solubilities. This suggested the possibility that the physical and chemical properties of the proteins synthesized might vary with the manner of their synthesis and it was decided to investigate this possibility.

PREPARATION AND ANALYSIS OF PROTEINS

The procedure employed in the preparation and separation of the various proteins was essentially the same in every case. The following account of the details of the preparation of the protein synthesized by pepsin + benzaldehyde was not changed in any essential except the omission of one constituent, in the preparation of the proteins synthesized by pepsin alone, by benzaldehyde alone or by benzene alone.

To 160 cc. of concentrated peptic digest at pH 4.0, 15 cc. of 10 per cent pepsin and 30 cc. of benzaldehyde, were added. The flask containing the mixture was thoroughly shaken, stoppered, and placed in a thermostat at 37° C. for 10 days. Twice a day during this period it was well shaken for 1 minute. At the end of 10 days the mixture was diluted to 2 litres. The synthesized protein was thus thrown out of solution. The suspension was now centrifuged, the supernatant fluid poured off, the precipitate re-suspended in distilled water and again centrifuged. This process was repeated until the wash water gave a negative or only faint reaction when tested for chlorides. It was assumed that at this stage all soluble nitrogenous material had also been removed. The water-washed precipitate contained some benzaldehyde in addition to protein and also adherent or insoluble resins resulting from the polymerization of benzaldehyde. The resins and benzaldehyde were completely removed by three washings with 90 per cent alcohol. The precipitate was then twice suspended in absolute alcohol and centrifuged. After removal of the alcohol with ether, the proteins were allowed to dry in the air at laboratory temperature. Where benzaldehyde was employed the flasks in which the synthesis was carried out were filled as completely as possible, to exclude air and thus minimize oxidation of the benzaldehyde to benzoic acid.

From 100 cc. of concentrated peptic digest, approximately 8 grams of protein was obtained with pepsin alone, about 12 grams from pepsin + benzaldehyde, 1 gram from benzaldehyde alone and 0.1 gram from benzene alone.

The results of analyses of the four proteins synthesized are summarized in Table VI.

TABLE VI. *Analyses of Proteins Synthesized by Various Means.*

Synthesizing Agent	Nitrogen in Air-Dried Protein Per Cent	Free Amino Nitrogen Total Nitrogen Per Cent	Free COOH Groups Total Nitrogen Per Cent
Pepsin alone	13.6	8.4	10.5
Pepsin + Benzaldehyde....	12.4	6.4	6.0
Benzaldehyde alone	13.9	5.9	12.6
Benzene alone	13.7

The similarity of the nitrogen content of the various proteins, shown in Table VI, indicates that no significant amounts of either benzaldehyde or benzene are included in the proteins synthesized through their agency. Their effects are probably exerted by adsorption of the constituents of the digest on the surface of the globules which are formed when benzaldehyde or benzene are shaken with digest.

The free amino-nitrogen was determined by the micro-method of Van Slyke, 2 cc. of a 1 per cent suspension being washed into the deaminating chamber with 1 cc. of water.

The free COOH groups were estimated, according to the method of Willstätter,¹⁶ by titration, in 90 per cent alcohol, with 90 per cent alcoholic potash, using thymolphthalein as indicator. Five cc. of the protein suspension was pipetted into 50 cc. of 98 per cent methylated ethyl alcohol and titrated with 0.1694 normal alcoholic potash. At the pH of the turning point of thymolphthalein the proteins went into solution leaving only a slight turbidity. The results in Table VI are given as equivalents of N/14 acid. They are averages of duplicates which do not vary more than 0.05 cc.

The results in Table VI show that these chemical properties, and therefore the constitution, of the proteins, vary with the means employed for synthesis. Difference in constitution is also demonstrated by the variation in their hydrolyzability by pepsin.

RESULTS OF HYDROLYSIS

In Table VII are given the results of hydrolysis of three different proteins. One per cent solutions of the synthesized proteins were made

¹⁶ Willstätter and Waldschmidt-Leitz, *Ber.*, 54B, 2988 (1921).

in dilute hydrochloric acid so that in each case a final pH of 1.5 was obtained. Pepsin was added to a concentration of 0.2 per cent. The temperature was maintained at 19° C. throughout the hydrolysis. Samples were removed at the times indicated and pipetted into trichloroacetic acid so that the final concentration of this acid was 2 per cent. The amount of hydrolysis was computed from determinations of total nitrogen before and after filtration. The protein synthesized by benzaldehyde alone is hydrolyzed much less rapidly than those which had been synthesized in the presence of pepsin.

TABLE VII. *Peptic Hydrolysis of 1 Per Cent Solutions of Proteins Synthesized by Different Means.*

Method of Synthesis	½ Hour	Per Cent Hydrolysis 1 Hour	2 Hours	3 Hours
Pepsin alone	19	36	49	56
Pepsin + benzaldehyde.....	51	64	75	75
Benzaldehyde alone	2	4	5	6

Proteins may also be characterized by their acid or base combining powers and the base combining capacity of the four proteins synthesized by different methods was accordingly determined.

In each case 0.25 grams of the protein, which had been air-dried for several weeks, was dissolved in 25 cc. of 0.2 *N* sodium hydroxide. Successive amounts of 0.2 *N* hydrochloric acid were added and the *e.m.f.* determined after each addition of acid. The Maloney hydrogen electrode¹⁷ was employed and suitable correction was made for temperature at each determination of hydrogen ion concentration.

BASE COMBINING CAPACITY OF SYNTHESIZED PROTEINS

In the calculation of base bound, the principle described by Cohn and Berggren¹⁸ was followed. The activity coefficients for the various ionic strengths were obtained by interpolation from the data given by Lewis and Randall¹⁹ and the values for pK_w at each temperature were interpolated from the data given by Michaelis.²⁰ The results are given in Tables VIII, IX, X and XI and Figure 4. All the significant data used in the computation of the base combining capacity are included in Table VIII. The base combining capacities given in Tables IX, X and XI were computed in exactly the same manner as in Table VIII but the

¹⁷ Maloney, *J. Phys. Chem.*, 25, 758 (1921).

¹⁸ Cohn and Berggren, *J. Gen. Physiol.*, 7, 45 (1924).

¹⁹ Lewis and Randall, "Thermodynamics and the Free Energy of Chemical Substances," New York and London, McGraw-Hill Book Co., 1923.

²⁰ Michaelis, L., "Hydrogen Ion Concentration," translated by Perlzweig, Baltimore, Williams & Wilkins, 1926.

TABLE VIII. *The Base Combining Capacity at Various Hydrogen-Ion Concentrations of Protein Synthesized by Pepsin.*
 Nitrogen content per gram = 136 mg.
 (0.25 gm. protein dissolved in 25 cc. 0.2N NaOH, and titrated with 0.2N HCl.)

Acid Added	E.m.f.	Temperature	pH Corrected for T	pK _w Corrected for T	pOH	α OH	Ionic Strength	Activity Coefficient NaOH	Con in Protein Solution	Con in NaOH Water Solution	Base Bound Per Gram	Equivalents $\times 10^5$
cc.	Volts	°C.										
0	1.0181	24.0	13.036	13.925	.837	.1455	0.20	0.77	.1887	.2000		113
2.0	1.0142	24.0	12.970	13.925	.903	.1250	0.185	0.79	.1582	.1704		121
4.0	1.0108	24.0	12.913	13.925	.960	.1096	0.17	0.80	.1373	.1448		91
6.0	1.0065	24.0	12.840	13.925	1.033	.0927	0.16	0.80	.1158	.1226		84
8.0	1.0036	24.0	12.791	13.925	1.083	.0826	0.15	0.80	.1033	.1030		— 4
10.0	.9988	24.0	12.709	13.925	1.165	.0684	0.14	0.80	.0854	.0857		+ 4
12.0	.9927	24.0	12.607	13.925	1.268	.0540	0.14	0.80	.0674	.0703		43
14.0	.9864	24.5	12.505	13.911	1.381	.0416	0.13	0.80	.0520	.0564		69
16.0	.9783	24.5	12.370	13.911	1.516	.0305	0.12	0.81	.0376	.0439		103
18.0	.9683	24.5	12.199	13.911	1.687	.0206	0.12	0.81	.0254	.0325		122
19.0	.9614	24.5	12.083	13.911	1.794	.0161	0.11	0.81	.0198	.0273		132
20.0	.9526	24.5	11.933	13.911	1.954	.0111	0.11	0.81	.0137	.0222		153
20.5	.9465	24.5	11.831	13.911	2.056	.0088	0.11	0.81	.0108	.0198		164
21.0	.9393	24.5	11.709	13.911	2.178	.0066	0.11	0.81	.0079	.0174		175
21.5	.9310	24.5	11.568	13.911	2.320	.0048	0.11	0.81	.0059	.0150		169
22.0	.9163	24.5	11.320	13.911	2.568	.0027	0.11	0.81	.0034	.0128		177
22.5	.8931	24.0	10.928	13.925	2.953	.0011	0.11	0.81	.0014	.0105		175
23.05	.8479	24.0	10.158	13.925	3.726	.0002	0.10	0.81	.0003	.0083		152
23.5	.7778	24.0	8.974	13.925	4.915	.000012	0.10	0.81	.0002	.0062		119
24.0	.7000	24.5	7.663	13.911	6.231	.0059	0.10	0.81	.0073	.0041		80
24.5	.6026	24.5	6.017	13.911	7.882	.013	0.10	0.81	.016	.0002		4

TABLE IX. *The Base Combining Capacity, at Various Hydrogen-Ion Concentrations, of Protein Synthesized by Benzene Alone.*

Nitrogen content per gm. of Protein = 137 mg.

(0.25 gm. protein dissolved in 25 cc. 0.2N NaOH and titrated with 0.2N HCl.)

Acid Added cc.	pH Corrected for Temperature	Base Bound Per Gram Equivalents $\times 10^5$
0.....	13.071	303
2.....	13.052	128
4.....	12.945	264
6.....	12.889	194
8.....	12.829	125
10.....	12.731	155
12.....	12.635	158
14.....	12.526	164
16.....	12.372	190
18.....	12.182	200
19.....	12.034	220
20.....	11.888	211
20.5.....	11.760	217
21.0.....	11.594	221
21.5.....	11.363	221
22.0.....	10.895	239
22.5.....	10.181	196
23.0.....	8.886	157
23.5.....	7.745	120
24.0.....	6.845	80
24.5.....	5.044	4

TABLE X. *The Base Combining Capacity at Various Hydrogen-Ion Concentrations of Protein Synthesized by Pepsin + Benzaldehyde.*

Nitrogen content per gm. of Protein = 124 mg.

(0.25 gm. protein dissolved in 25 cc. 0.2N NaOH and titrated with 0.2N HCl.)

Acid Added cc.	pH Corrected for Temperature	Base Formed Per Gram Equivalents $\times 10^5$
0.....	13.120	310
2.....	13.082	205
4.....	13.023	172
6.....	12.951	155
8.....	12.845	192
10.....	12.768	162
12.....	12.671	129
14.....	12.564	126
16.....	12.432	144
18.05.....	12.264	145
19.....	12.160	145
20.....	12.004	160
20.5.....	11.909	164
21.....	11.808	164
21.55.....	11.659	167
22.....	11.486	164
22.5.....	11.181	162
23.....	10.668	147
23.5.....	9.577	118
24.....	7.852	78
24.5.....	6.318	4

TABLE XI. *The Base Combining Capacity at Various Hydrogen-Ion Concentrations of Protein Synthesized by Benzaldehyde Alone.*

Nitrogen content per gm. of Protein = 139 mg.

(0.25 gm. protein dissolved in 25 cc. 0.2N NaOH and titrated with 0.2N HCl.)

Acid Added	pH Corrected for Temperature	Base Formed Per Gram Equivalents $\times 10^5$
cc.		
0.....	13.145	165
2.....	13.058	192
4.....	12.983	178
6.....	12.895	210
8.....	12.832	153
10.....	12.751	137
12.....	12.642	149
14.....	12.526	134
16.....	12.376	165
18.....	12.210	167
19.....	12.088	178
20.....	11.929	186
20.5.....	11.834	188
21.....	11.682	195
21.5.....	11.512	195
22.....	11.250	194
22.5.....	10.802	182
23.05.....	9.996	157
23.5.....	8.756	118
24.0.....	7.347	80
24.5.....	6.145	4

details have been omitted from the tabulation. They show distinct and consistent differences in base combining power throughout the whole range of hydroxyl-ion concentrations observed. The highest base combining capacity is exhibited by the protein synthesized by emulsifying with benzene alone. This protein binds $239 \text{ equivalents} \times 10^5$ of base per gram at pH 10.89. The corresponding maximum values for the protein synthesized by emulsification with benzaldehyde is 195 equivalents $\times 10^5$ at pH 11.68 with benzaldehyde + pepsin it is 167 equivalents $\times 10^5$ at pH 11.66 and with pepsin alone it is 177 equivalents $\times 10^5$ at pH 11.34.

The order of the base combining capacities coincided with the solubilities of the protein in the range between pH 5.0 and pH 8.0. All four proteins were soluble to an extent of at least 1 per cent in alkaline solutions down to pH 8.0 but the proteins prepared by pepsin alone and by pepsin + benzaldehyde were flocked out of solution at about pH 7.6. Those prepared by benzene and by benzaldehyde alone were quite soluble at neutrality and were not thrown out of solution until the benzaldehyde-protein solution reached pH 6.0 and the benzene-protein solution pH 5.0. The order of magnitude of the first maximum in base

combining capacity for all these artificial proteins corresponds to that observed for casein by Robertson and by Cohn and Berggren.¹⁸

Figure 4 shows that after the first maximum is reached, with increasing alkalinity, the base combined decreases very rapidly, to rise rapidly again to a second undetermined maximum. A possible explana-

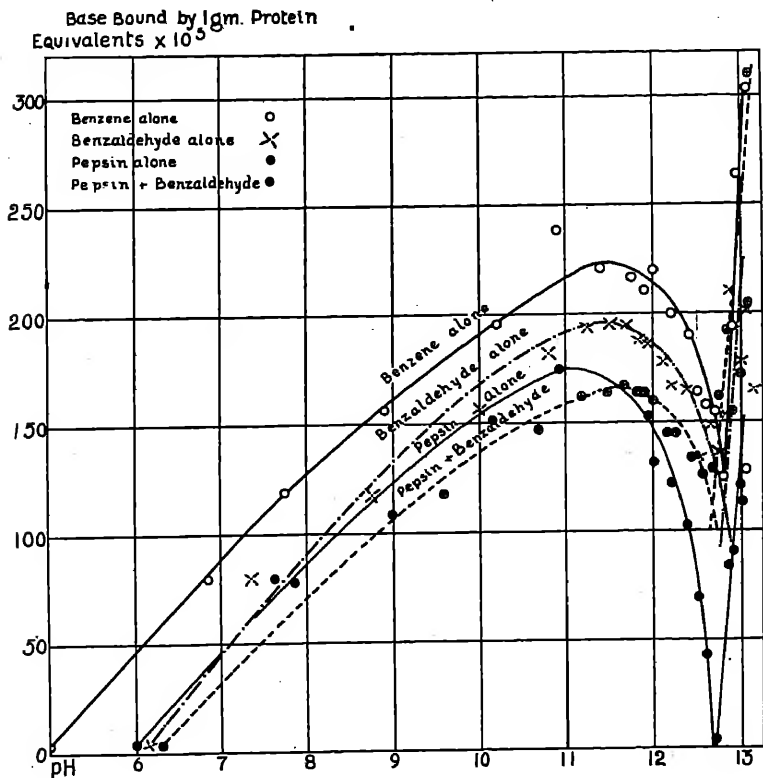


FIG. 4.—The base-combining capacities of proteins synthesized by different means.

tion for this curious behavior was suggested by Professor R. A. Gortner, in conversation with the authors, to be due to an effect exerted by the first formation of neutral salt in the hitherto salt-free solutions. The differences in the transient minima obtained with the different proteins could be explained, on this basis, by differences in the completeness with which salt had been washed out during their preparation for analysis.

SUMMARY

- (1) The effect of the presence of emulsions during peptic synthesis of protein has been investigated.
- (2) Emulsions formed with benzaldehyde, benzoic acid, benzene, toluene and chloroform have a definite accelerating effect on synthesis.
- (3) Emulsions formed with oleic acid or olive oil show no accelerating effect on synthesis.
- (4) The presence of talc powder, kieselguhr and barium sulphate slightly accelerates peptic synthesis.
- (5) Under certain conditions, the presence of effective emulsions causes an augmentation of the equilibrium amount of protein synthesized, but under optimum conditions for synthesis no such augmentation of the amount synthesized is effected although an acceleration of the rate of synthesis always occurs.
- (6) The effective emulsions are found to induce some synthesis even in the absence of enzyme.
- (7) It is found that the proteins synthesized vary in their physical and chemical properties according to the means which have been employed to effect synthesis.

*University of Toronto,
Toronto, Ontario, Canada.*

EMULSIONS AND THE EFFECT OF HYDROGEN-ION CONCENTRATION UPON THEIR STABILITY

By JOHN C. KRANTZ, JR.,* AND NEIL E. GORDON

INTRODUCTION

Since the publication of the work of Harkins¹ and Langmuir² on the structure of liquid surfaces at a liquid-liquid interface, the orientation theory of emulsions and emulsification has been the object of much investigation and experimental work. Newman³ working with Bancroft in 1914 observed that when benzene and water were emulsified using sodium oleate as the emulsifying agent the hydrocarbon formed the inner phase, whereas when oleic acid salts of metals with a higher valence, *e.g.*, magnesium oleate, were employed, the water becomes dispersed thru the benzene, the latter becoming the continuous phase. These observations were substantiated by Harkins and are in accord with his theoretical deductions of the nature of emulsifying agents.

Harkins and his co-workers believe that the stability of the dispersed particles in an emulsion is accomplished by the orientation of the molecules at the interface with the medium of dispersion. An emulsifying agent in the light of Harkins' theory should be a compound having a polar and non-polar group in the molecule. Hence the alkali salt of a fatty acid such as oleic or stearic acid meets this requirement. As emulsions are systems of two immiscible liquids, the emulsifying agent must serve to reduce the abruptness of the transition between the two liquids. An emulsion, according to Harkins, is stable when the molecules of the film of the emulsifying agent fit the curvature of the emulsified particle. Accordingly sodium oleate reduces the free energy between benzene and water and thus induces the emulsification of the benzene in the water, whereas with magnesium oleate the film of the emulsifying agent fits better the water particle and this in turn becomes dispersed in benzene.

In the preparation of medicinal emulsions gum arabic and tragacanth

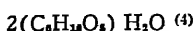
* This article is taken from the work presented by John C. Krantz, Jr., to the Graduate School of the University of Maryland in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

¹ Harkins et al., *J. Am. Chem. Soc.*, 39, 353, 541 (1917).

² Langmuir, *J. Am. Chem. Soc.*, 39, 1848 (1917).

³ Newman, *J. Phys. Chem.*, 18, 34 (1914).

are the most generally used emulsifying agents and yield very stable milk-like emulsions. Acacia in all of the generally used medicinal emulsions invariably produces an emulsion of the oil-in-water type and as this type of emulsion is the more palatable, the gum serves well for the preparation of medicinal emulsions. In general treatises on *Materia Medica* and medicinal chemistry this gum is referred to as the sodium, potassium and calcium salts of arabic acid. The general texts on pharmacy and pharmaceutical chemistry have assigned the following formulas to arabic acid:



The gum itself contains varying amounts of sodium and potassium and certain samples are practically pure calcium arabate.

Not much work has been done on the determination of the structure of arabic acid. Beilstein gives the following empirical formula for the acid which has been dried at 100°C .:— $C_{18}H_{18}O_8(?)$. Neubauer⁸ prepared the calcium and potassium salts and found them to have the following compositions:— $\text{CaO} : 2C_{12}H_{20}O_{10}K_2O \cdot (C_{12}H_{22}O_{11})_3(?)$. Hackmeyer⁹ found the lead, copper and barium salts to have the following compositions: $2PbO \cdot 3C_{12}H_{20}O_{10}$, $\text{CuO} \cdot C_{12}H_{20}O_{10} \cdot C_{12}H_{22}O_{11}$, $\text{BaO} \cdot 2C_{12}H_{20}O_{10}$. O'Sullivan¹⁰ prepared calcium and barium salts of the acid which he analyzed and to which he assigned the following formulas: $C_{80}H_{142}O_{74} \cdot \text{Ca}$ and $C_{89}H_{142}O_{74} \cdot \text{Ba}$. By hydrolyzing arabic acid with diluted sulfuric acid, the same investigator obtained a series of acids having 23 to 71 carbon atoms in the molecule. Bechamp¹¹ obtained the di and tetra nitro substitution products of arabic acid by treating gum arabic with hot nitric acid. These compounds were amorphous and were assigned the compositions $C_{12}H_{18}(\text{NO}_2)_2O_{10}(?)$ and $C_{12}H_{18}(\text{NO}_2)_4O_{10}(?)$ respectively. Likewise the same investigator succeeded in acetylizing the gum with acetic anhydride at 105°C . and obtained an amorphous insoluble compound of the composition $C_{12}H_{18}(C_2H_3O)_4O_{10}(?)$.

This summary indicates that little is known about the structure of arabic acid, but from the work that has been done, the compound seems

⁸ Caspari, "Treatise on Pharmacy for Students and Pharmacists," New York, Lea and Febiger, 1926, p. 737.

⁹ Simon, "Manual on Chemistry," New York, Lea and Febiger, p. 510.

¹⁰ Dorland, "American Pocket Medical Dictionary," Philadelphia, W. B. Saunders, 1922, p. 72.

¹¹ Remington, "Practice of Pharmacy," Philadelphia, J. B. Lippincott, 1926, p. 736.

¹² Neubauer, *Jahresbericht*, 624 (1854).

¹³ Heckmeyer, *Jahresbericht*, 484 (1858).

¹⁴ O'Sullivan, *J. Chem. Soc.*, 45, 54 (1884).

¹⁵ Bechamp, *Jahresbericht*, 521 (1860).

to be similar to familiar carbohydrates in structure or to the carboxylic acids obtained by mild oxidation of the carbohydrates.

Tragacanth, another gum-like substance, which is employed like acacia to prepare medicinal emulsions, invariably produces emulsions of the oil-in-water type, although this substance like acacia consists of the calcium salt of a complex organic acid. The principal acid constituent of this gum is bassoric acid, to which Sullivan¹² assigned the formula $\text{H.C}_{24}\text{H}_{84}\text{O}_{20} \cdot \text{H}_2\text{O}$.

As arabic acid is obtained from acacia in a pure form far more readily than bassoric acid is obtained from tragacanth, this substance was selected as the typical acid for study.

In view of Harkins' and Newman's experiences with sodium and magnesium oleates, it was decided to investigate the emulsifying properties of acacia and tragacanth in order to determine the effect of a univalent or divalent metal in the emulsifying agent. It will be recalled that as the oleates of sodium and magnesium have but one polar group in the molecule, namely the respective metallic atoms, the sodium and magnesium salts of arabic acid probably contain the polar hydroxyl groups (as indicated by their union with acetic anhydride and nitric acid) in addition to the polar metallic atoms. In addition to investigating the character of the emulsions formed by the univalent and divalent salts of these acids, it was thought necessary to study the influence of changes of hydrogen-ion concentration upon the stability of emulsions made with acacia and tragacanth and to determine the influence of this factor upon the two emulsifying agents.

The influence of changes of hydrogen-ion concentration upon the emulsions prepared with acacia and tragacanth of the oil-in-water type was interesting enough to warrant studying this influence upon emulsions of the water-in-oil type. It was found impossible, however, to employ the divalent salts of arabic acid for this purpose, as will be observed from the character of these emulsifying agents; therefore to prepare these emulsions magnesium oleate was employed.

Although for years, in medicinal emulsions, the presence of acids was considered detrimental to the stability of emulsions, whereas alkalies¹³ have been looked upon as stabilizers, on account of their union with vegetable and animal oils to form soap, very little work has been done to study the influence of changes of actual hydrogen-ion concentration upon emulsions and emulsifying agents. The importance of the stability of emulsions and those agents which influence this, cannot be over-estimated; not only has this field an important pharmaceutical significance, but manufacturers of salad dressings and food

¹² Sullivan, *Pharm. Zentralhalle*, 62, 582 (1921).

¹³ Tfol, *Pharm. J.*, 6, 1228 (1898).

products in general, many of which are emulsion-like in nature, are calling upon the chemist to interpret and rectify the instability of their products.

The salient problems for study then in this investigation are; first, the study of the type of emulsions produced by salts of arabic acid and related compounds; second, the study of the influence of changes in hydrogen-ion concentration upon emulsions prepared with these emulsifying agents of the oil-in-water type, and third, a study of the influence of changes in hydrogen-ion concentration upon water-in-oil emulsions.

EXPERIMENTAL

MATERIALS AND APPARATUS

1. *Arabic Acid*.—This compound was obtained by slightly acidifying a solution of acacia in water with hydrochloric acid, dialyzing to remove soluble chlorides and precipitating the arabic acid from its aqueous solution by means of alcohol. Merck's Arabin was also employed.

2. *Magnesium Arabate*.—The compound was prepared by boiling a solution of arabic acid in water with an excess of magnesium carbonate, filtering and evaporating the filtrate to dryness over a water-bath. Upon ignition the compound yielded 2.4 per cent of magnesium oxide.

3. *Sodium Arabate*.—The calcium present in a solution of acacia in water was precipitated by the addition of sodium carbonate and the solution brought to the neutral point. The filtrate was evaporated to dryness over a water-bath.

4. *Ferric Arabate*.—A 20 per cent solution of acacia in water was treated with ferric chloride solution, added drop by drop until a gel was obtained of uniform reddish-brown color and a stiff consistency. The iron (Fe) content of the gel was determined by treatment with hydrochloric acid and potassium iodide in the usual manner and titrating the liberated iodine with sodium thiosulphate. The gel contained 0.39 per cent of iron.

5. *Lead Arabate*.—A solution of acacia in water was treated with a solution of lead subacetate $\text{Pb}_2\text{O}(\text{CH}_3\text{COO})_2$ equivalent to 18 per cent of metallic lead, as long as precipitation occurred. The precipitate was washed free of soluble lead salts and dried to a constant weight at 100°C . The lead content was determined by dissolving about one gram of the lead arabate in diluted nitric acid and precipitating the lead as carbonate by means of sodium carbonate, washing, filtering and igniting, weighing the lead as the monoxide. The compound contained 40.25 per cent PbO .

6. *Sodium Valerate*.—This was obtained by neutralizing valeric acid with sodium bicarbonate and evaporating the solution to the crystallizing point.

7. *Magnesium Valerate*.—This was prepared by dissolving magnesium borings (for Grignard reaction) in a mixture of valeric acid and water. After the reaction was complete the solution was evaporated to the crystallizing point.

8. *Zinc and Ammonium Valerates*.—These compounds were used from a supply of the salts of medicinal purity in the laboratory.

9. *Calcium Gluconate*.¹⁴

10. *Cadmium i-galactonate*.¹⁵

11. *Magnesium Dioxystearate*.¹⁶

12. *Gums*.—The acacia and tragacanth employed met the requirements prescribed by the United States Pharmacopoeia for these substances.

13. *Oils and Water*.—The mineral oil employed was the commercial "Nujol" and the cottonseed and olive oils met the requirements of the United States Pharmacopoeia. Distilled water pH 5.8 to 6.4 was employed.

14. *APPARATUS*.—The special apparatus employed in this investigation consisted of a hydrogen-ion determination outfit with a type K potentiometer and a Wilson hydrogen electrode, a Du Noüy tensiometer and a Donnan pipette which is shown in Figure 1.

EMULSIFYING AGENTS

Certain Oleates as Emulsifying Agents.—In alignment with the work of Harkins and Newman several emulsions were prepared of mineral oil using sodium oleate, magnesium oleate, calcium oleate, manganese oleate, cobaltous oleate, nickelous oleate and aluminum oleate as emulsifying agents. Similar to the observations of Newman and Harkins with benzene, the sodium oleate yielded a dispersion of oil in water, whereas the dispersion prepared with the oleates of the divalent metals and aluminum gave emulsions of the water-in-oil type.

Valerates as Emulsifying Agents.—Considering the fact that in the simplest formula assigned to arabic acid, $C_5H_{10}O_{11}$, five carbon atoms are present in the molecule, an acid of the methane series with 5 carbon atoms in the molecule was selected for study. This compound, valeric acid, is of definite composition and gave an excellent starting point for study. Emulsions of mineral oil prepared with the sodium and

¹⁴ E. Fischer, *Guide de Preparations Organiques*, p. 86.

¹⁵ Kiliani, *Ber.*, 18, 1552 (1885).

¹⁶ Groger, *Ber.*, 18, 1268 (1885).

ammonium salts of this acid were of the oil-in-water type and permitted slight dilution with water, but could not be diluted with oil at all. The procedure employed was to place 1 gram of the salt in a mortar and add 5 cc. of oil and 5 cc. of water and triturate briskly until the mixture was homogeneous. These emulsions were not very stable, and upon standing 12 to 18 hours the oil and water separated, yet upon agitation the oil was again dispersed thru the water.

The magnesium and zinc salts of valeric acid, using the same quantities and procedure as directed in the foregoing experiment, yielded emulsions of the water-in-oil type. As the salts of valeric acid were found to possess far less emulsifying power than the salts of oleic acid, more difficulty was experienced in determining whether the emulsion was of the oil-in-water type or vice versa. In order to overcome this difficulty the water was colored slightly by an organic pigment, cudbear, which is insoluble in the oil. The continuity of this pigment in the emulsion indicated that water was the outer or continuous phase, whereas the discontinuity of the red color showed that the water was dispersed in the oil.

Arabates as Emulsifying Agents.—Having demonstrated the capacity of divalent metals when combined with the five carbon atoms valeric acid to form water-in-oil emulsions, several emulsions were prepared using different salts of arabic acid. Arabic acid, sodium and magnesium arabates gave stable emulsions of the oil-in-water type. The procedure employed was to triturate 10 cc. of the oil with 2.5 grams of the emulsifying agent and add in one portion 5 cc. of water. Then after brisk trituration and the formation of the emulsion nucleus, the remainder of the water was added slowly with continued trituration until a 40 cc. volume was obtained. Thus in each case a 25 per cent oil-in-water emulsion was prepared.

The iron gel prepared with arabic acid gave an emulsion of the oil-in-water type, by using 15 grams of the gel, 5 cc. of water and 10 cc. of oil and briskly triturating until emulsification occurred. This then was diluted with water until 40 cc. was obtained. These emulsions were stable over several weeks but separated a creamy layer upon the surface as do typical emulsions prepared with gum arabic.

With lead arabate as an emulsifying agent, the oil could not be emulsified in water, and likewise it was impossible to prepare an inverted emulsion using this emulsifying agent. When 1.5 grams of the salt was briskly triturated with 5 cc. of oil and 5 cc. of water, the oil quickly separated to the surface of the mixture. Lead arabate was practically insoluble in water and in oil.

These experiments indicate that although the magnesium and zinc salts of valeric acid yield emulsions of the water-in-oil type, the mag-

nesium and iron salts of arabic acid gave the normal oil-in-water emulsion.

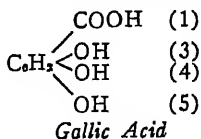
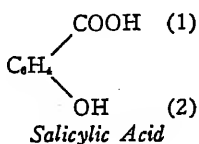
Compounds Assumed to be Related to Arabic Acid as Emulsifying Agents.—Having shown that the divalent and trivalent salts of arabic acid produce emulsions of the oil-in-water type, the next step in experimental work was to select a number of acids of definite structure having hydroxyl groups in the molecule and determine the nature of the emulsions prepared with their divalent salts.

The salts of the following acids were employed:

$\text{OH} \cdot \text{CH}_2(\text{CH} \cdot \text{OH})_4 \cdot \text{COOH}$ *i-Galactonic Acid*
(a mixture of *d* and *l*-Galactonic acids)

$\text{OH} \cdot \text{CH}_2(\text{CH} \cdot \text{OH})_4 \cdot \text{COOH}$ *d-Gluconic Acid*

$\text{CH}_3(\text{CH}_2)_7 \cdot \text{CH} \cdot \text{OH} \cdot \text{CH} \cdot \text{OH} \cdot (\text{CH}_2)_7 \cdot \text{COOH}$ *Dihydroxystearic Acid*



The sodium salts and also a salt of a divalent metal were prepared from each of these acids and when employed as emulsifying agents the following observations were made.

1. *Gluconic Acid*.—The sodium salt of gluconic acid like the sodium salt of valeric acid produced emulsions with oil of the oil-in-water type. Five grams of the compound was necessary to disperse 10 cc. of oil through 10 cc. of water, yielding an emulsion which was very viscid, yet upon standing for a period of an hour it began to separate.

The calcium salt of gluconic acid was treated in the following manner: One gram of the salt was rubbed with 5 cc. of oil, and water (5 cc.) was gradually added with brisk trituration. An emulsion of the water-in-oil type was obtained. A microscopic examination of the emulsion showed the particles of water to be rather large, yet the emulsion was viscous and stable over a period of several weeks.

Reversing the procedure of mixing, and employing the same emulsifying agent, an emulsion was obtained of the oil-in-water type. The particles of oil emulsified were like the particles of the water in the previous experiment, somewhat larger than those found in most emulsions. Upon standing the oil and water separated, but not completely and evidence of an emulsion nucleus remaining permanent was shown by the fact that upon simple agitation, the emulsion was restored to its normal condition. It is of special interest to note the value of viscosity

in determining the phases of emulsions of this type; those in which the viscous mineral oil was the external phase were quite viscous, whereas those in which the oil formed the inner phase had a low viscosity.

2. *i-Galactonic Acid*.—The sodium salt of *i*-galactonic acid, like the sodium salt of gluconic acid, produced dispersions of the oil-in-water type, whereas the cadmium salt of this compound, like the calcium salt of gluconic exhibited the capacity of dispersing oil in water or water in oil. Efforts were made to prepare the aluminum and ferric compounds of gluconic and *i*-galactonic acids, but neither of these acids would combine with aluminum or trivalent iron.

3. *Dioxystearic Acid*.—Having observed the capacity of each of these acids to form oil-in-water and water-in-oil emulsions, the next compound prepared and studied was dihydroxystearic acid, which has but two hydroxyl groups in a molecule containing a chain of seventeen carbon atoms. The sodium salt of dihydroxystearic acid produced very stable emulsions of the oil-in-water type, whereas the magnesium salt of this acid produced emulsions of the water-in-oil type. Under no circumstances was it found possible to prepare emulsions with magnesium dioxystearate of the oil-in-water type. Thus the dihydroxyl acid combined with a divalent metal behaved like a normal stearic acid salt, whereas the polyhydroxyl compounds, *i*-galactonic and gluconic acids, showed the unusual property of emulsifying water in oil and also oil in water.

4. *Salicylic Acid*.—Having observed the influence of hydroxyl groups in the straight chain acids, the next step was to study the influence of the introduction of hydroxyl groups into acids containing cyclic groups. Accordingly sodium salicylate was found to produce emulsions which were unstable, but always of the oil-in-water type. Calcium salicylate on the other hand produced rather stable emulsions of water in oil, although the water particles were quite large. Employing the procedures described under the gluconic acid salts, attempts were made to prepare emulsions of the oil-in-water type, and although the particles of oil were exceedingly large and rapidly separated, there is undoubtedly some tendency on the part of calcium salicylate to form emulsions of the oil-in-water type.

5. *Gallic Acid*.—The sodium salt of gallic acid, although a poor emulsifying agent, was shown to invariably produce dispersions of the oil-in-water type. The calcium salt of gallic acid produced rather stable emulsions of the water-in-oil type and also served, to a greater degree than did calcium salicylate, to produce emulsions of the oil-in-water type. From the observations made with gluconic and *i*-galactonic acids in the form of their divalent salts, this is the behavior that might have been anticipated.

TABLE I. *Summary of Results.*

Emulsifying Agent	Type of Emulsion		
	Oil-in-Water	Amphoteric Properties	Water-in-Oil
Sodium oleate	X		
Magnesium oleate			X
Calcium oleate			X
Cobaltous oleate			X
Nickelous oleate			X
Manganese oleate			X
Aluminum oleate			X
Arabic acid	X		
Sodium arabate	X		
Magnesium arabate	X		
Ferric arabate	X		
Ammonium valerate	X		
Sodium valerate	X		
Magnesium valerate			X
Zinc valerate			X
Sodium gluconate	X		
Calcium gluconate		X	
Sodium α -galactonate	X		
Cadmium α -galactonate		X	
Sodium dihydroxystearate	X		
Magnesium dihydroxystearate			X
Sodium salicylate	X		
Calcium salicylate		X	
Sodium gallate	X		
Calcium gallate		X	
Lead arabate	Did not serve as an emulsifying agent		

Table I indicates that the simple hydrocarbon chain acids when attached to a univalent metal produce emulsions of the oil-in-water type and inverted emulsions when the metallic ion is divalent or trivalent. When, however, polar hydroxyl groups are introduced into the molecule, this condition does not hold as indicated by the character of emulsions prepared with magnesium and ferric arabates and other related compounds.

Throughout this investigation water of definite hydrogen-ion concentration pH 5.8 to 6.4 was employed. Having established the character of emulsions produced when the salts of compounds of the nature of arabic acid are employed as emulsifying agents, the next series of investigations was directed to determine the influence of changes of hydrogen-ion concentration upon the oil-in-water emulsions when acacia and tragacanth were employed as emulsifying agents.

METHOD OF STUDY OF THE INFLUENCE OF HYDROGEN-ION CONCENTRATION UPON OIL-IN-WATER EMULSIONS

Acacia and tragacanth were the two emulsifying agents studied with emulsions of cottonseed and mineral oils (Nujol). Solutions of various

hydrogen-ion concentrations were prepared by mixing standard sodium hydroxide solution and standard hydrochloric acid respectively, with water in various dilutions. The pH of the solutions was determined by the electrometric method. These solutions served as the diluents in the preparation of 25 per cent emulsions of the oil. The total volume of the emulsion in each case was 40 cc.

In the emulsions made with tragacanth 0.5 gram of the tragacanth was employed, whereas 2.5 grams of acacia was used in the emulsions prepared with this substance. The emulsions were prepared by trituration in a mortar—mixing the emulsifying agent with the oil and adding the aqueous solutions—as far as possible uniform conditions of temperature, pressure and time of trituration and rapidity of dilution were kept constant.

The finished emulsions were stored at room temperature in small graduated cylinders and their permanence studied. Complete separation of the emulsified portion from the aqueous layer is termed separation, creaming or partial separation, which is easily reincorporated by simple agitation, is not regarded as separation.

The following observations were made upon the changes of pH* in oil-in-water emulsions.

TABLE II. *Cottonseed Oil Emulsified with Acacia.**

Degree of separation in cc. in different time periods.

No.	pH	Days						
		1.5	7	14	22	30	37	50
1	0.4		14	c.s.	c.s.	c.s.	c.s.	c.s.
2	0.9		5	10	10	10	10	10
3	1.4		2	5	5	5	5	5
12	8.7							1
13	10.5							3
14	11.8		c.s.	c.s.	c.s.	c.s.	c.s.	c.s.
15	12.5		c.s.	c.s.	c.s.	c.s.	c.s.	c.s.
16	13.2	c.s.†	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.

* Emulsions 4, 5, 6, 7, 8, 9, 10, 11—pH 2.0, 2.94, 3.85, 4.45, 5.1, 6.3, 6.9 and 7.85 showed no separation over this period. This policy is followed in subsequent tables, where no separation took place.

† c.s. = complete separation.

After this period there was no change noticed for weeks and the emulsions when discarded months later retained the relationship to permanence as indicated by the table. The creaming effect reached a maximum of 27 cc. in about 22 days. However, as mentioned before this phenomenon was not considered as separation.

* The pH measurements indicate the pH of the outer phase without the emulsifying agent prior to mixing. The pH of the finished emulsions may be observed from Table IX.

TABLE III. *Cottonseed Oil Emulsified with Tragacanth.*

Degree of separation in cc. in different time periods.

No.	pH	Hours	Days										
		12	2	5	10	17	25	33	41	58	100	130	
1	0.6	2	3	4	5	8	10	
2	1.05	1	1.5	2	6	10	
3	1.6	1	8	20	
4	2.65	5	16	25	
5	3.4	2	5	11	17	c.s.	
6	4.2	2	3	6	8	11	12	c.s.	
7	5.2	2	5	10	15	23	c.s.	
8	6.25	1	5	8	11	17	c.s.	
9	7.8	1	5	22	30	30	35	35	c.s.	
10	8.5	2	4	10	11	16	22	c.s.	
11	9.45	3	8	10	16	21	c.s.	
12	10.35	25	7	21	25	26	26	26	c.s.	
13	11.8	25	25	25	27	27	27	c.s.	
14	12.4	light yellow	deeper yellow	deeper yellow	3	c.s.*	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	
15	13.35	deep yellow	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	

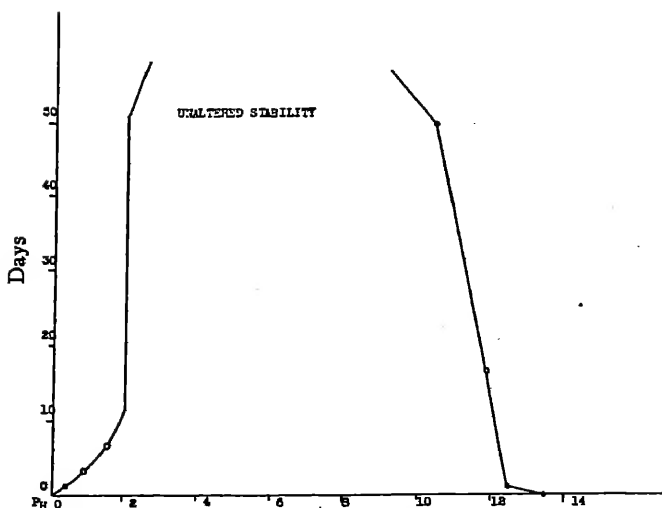
* c.s. = complete separation.

TABLE IV. *Mineral Oil Emulsified with Acacia.*

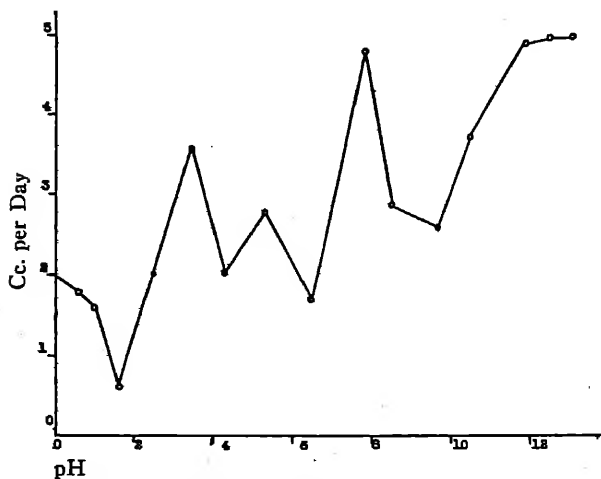
Degree of separation in cc. in different time periods.

No.	pH	Hours	Days											
			3	1	2	4	7	12	19	27	35	43	60	100
1	0.6	1	2	2	2	3	20	26	27	27	27	
2	1.05	1	1	1	2	3	3	3	3	3	
											deeper	deep	deep	
13	11.8	low	low	low	low	
14	12.4	..	1	1	2	25	25	25	26	26	26	26	26	
15	13.35	1.5	2	c.s.*	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	
		(yel-	(deeper											
		low)	yellow)											

* c.s. = complete separation.



GRAPH No. 1.—Stability of Emulsions with Acacia and Their Hydrogen-Ion Concentrations.



GRAPH No. 2.—Stability of Emulsions with Tragacanth and Their Hydrogen-Ion Concentrations.

TABLE V. *Mineral Oil Emulsified with Tragacanth.*

Degree of separation in cc. in different time periods.

No.	pH	Hours		Days									
		12	1	3	6	11	17	25	33	41	60	100	
1	0.6	2	4	8	12	18	21	22	22	
2	1.05	0.5	5	10	17	21	22	23	24	27	
3	1.6	2	2	9	22	
4	2.65	1	3	5	8	12	19	25	
5	3.4	2	7	12	17	21	22	26	28	
6	4.2	2	3	6	10	10	20	26	
7	5.2	4	8	11	15	16	17	21	26	
8	6.25	1	5	10	18	23	26	28	29	
9	7.8	3	6	10	16	21	23	25	28	
10	8.5	2	8	11	18	20	21	23	24	
11	9.45	10	24	28	30	30	31	38	
12	10.35	7	10	16	20	22	25	38	
13	11.8	25	25	30	30	30	30	30	30	
14	12.4	..	1	2	c.s.*	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	
		yel-low	(deep-er yellow)	(deep-er yellow)									
15	13.35	2	10	20	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	c.s.	
		(deep yellow)											

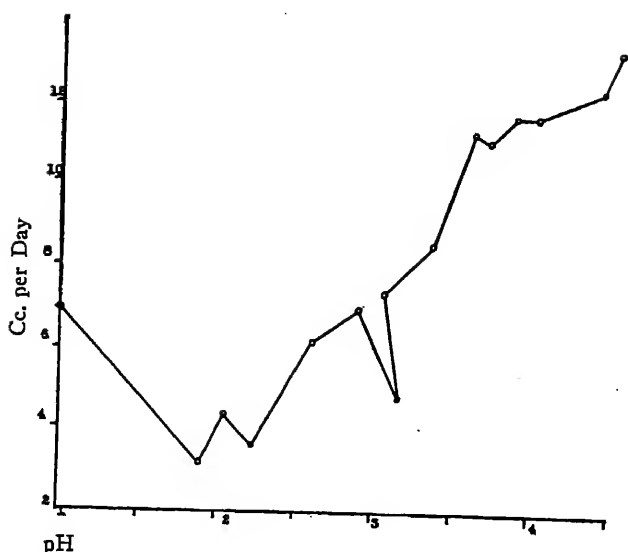
* c.s. = complete separation.

Tables II and IV indicate that there is a long range of hydrogen-ion concentration over which the emulsions made with acacia are stable and further that these results are the same with a vegetable or a mineral oil. With tragacanth the most stable point of hydrogen-ion concentration of the external phase is from pH 1.8 to 2.3. The average stability of the experiments recorded in Tables III to V can be easily observed from Graphs 1 and 2.

The ordinates of Graph 2 represent the amount of separation per day over a period of 60 days and are multiplied by ten to eliminate decimals.

Graph 2 indicates that over a large range of pH the stable point is approximately pH 1.90. With this in mind other series of emulsions were prepared covering the acid range of pH with smaller variations in order to determine the exact stable point. These results with tragacanth are recorded in Table VI and the average stability over a period of 20 days is plotted in Graph 3. The hydrogen-ion determinations were made upon the external phase of the emulsions prior to mixing.

Three series of acacia emulsions were prepared over the same range of hydrogen-ion concentration, but little or no variation in their stability was observed. Those prepared with an external phase pH 1 seemed to separate a creamy layer more readily and ultimately (after about 20 days) separate to the extent of 5 to 10 cc.



GRAPH No. 3.—Stability of Emulsions with Tragacanth and Their Hydrogen-Ion Concentrations.

The cc. of separation per day was multiplied by ten to construct the ordinates of this graph.

TABLE VI. *Cottonseed Oil Emulsified with Tragacanth.*
Degree of separation in cc. in different time periods.

No.	pH	Days										
		4	6	8	10	12	14	17	21	25	32	38
1	1	1.5	1.5	1.5								
2	1.92	2	2	3	4	5	6.5	8
3	2.08	1	2	3.5	5	8	11
4	2.22	0.5	1	2.5	4
5	2.4	1	3	8	13
6	2.65	0.5	1	2	3
7	2.94	1	2	2	5	11	14
8	3.02	1	1	2	2.5	4	4	5	9	12
9	3.15	0.5	0.5	1	1	2	3	3	5
10	3.3	..	1.5	3	4	6	7	9	11	13	16	18
11	3.6	1	1.5	2	3	4	6	8	10
12	3.7	..	2	4	7	9	11	13	17	19	22	23
13	3.82	..	2.5	4	7	9	11	13	17	19	22	24
14	4	..	3	5	9	12	15	17	20	21	22	23
15	4.1	..	3	7	13	17	19	22	23	24	24	25
16	4.4	2	5	9	13	17	19	22	25	26	26	27
17	4.5	1	5	10	15	17	19	21	23	24	25	26
		2	8	21	27	28	28	28	29	29	29	29

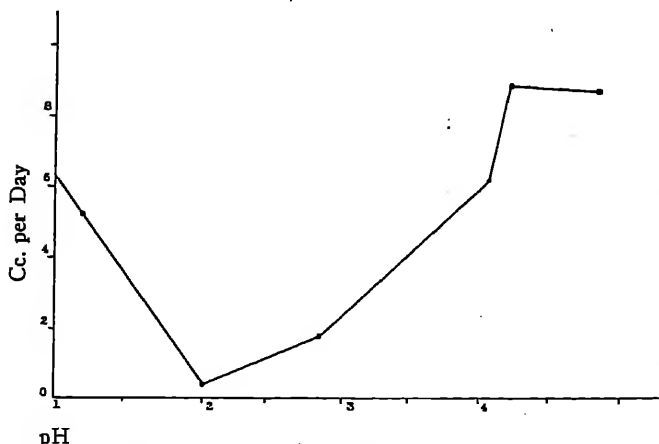
The effect of sodium chloride solutions in various concentrations upon these emulsions was studied in order to determine the influence of the sodium ion. When studied in concentrations from 1 *N* to 10^{-6} *N*, it was found that none of these concentrations of sodium chloride solution affected the stability of the emulsion. Within 4 or 5 days all emulsions prepared with acacia separated a creamy layer of 25-27 cc. and remained in that condition for more than 150 days, the effect of all concentrations of sodium chloride used showing the same results.

The influence of sodium chloride solutions upon tragacanth emulsions of mineral oil is shown by the following table:

TABLE VII. *Mineral Oil Emulsified with Tragacanth in Various Concentrations of Sodium Chloride Solution.*

Degree of separation in cc. in different time periods.

No.	Normality	Days						
		5	11	19	27	25	53	100
1	1	3	5	8	10	15
2	0.1	1	2	5	8	10
3	10^{-2}	1	1	2	4	8
4	10^{-3}	..	1	5	9	15	20	27
5	10^{-4}	..	1	3	5	8	15	27
6	10^{-5}	..	10	10	15	17	23	27
7	10^{-6}	10	15	21	23	25	27	27



GRAPH No. 4.—Stability of Emulsions with Tragacanth and Their Hydrogen-Ion Concentrations: Sulfuric Acid Used.

In order to study the influence of the hydrogen ion produced from another source, solutions of sulfuric acid were prepared and the same type of emulsions were made from these. With acacia little

change was noticed except in the emulsion of a pH 1.2 where partial separation occurred. The results obtained with tragacanth may be observed from Table VIII and Graph 4.

TABLE VIII. *Mineral Oil Emulsified with Tragacanth in the Presence of Sulfuric Acid.*

Degree of separation in cc. in different time periods.

No.	pH	Days					
		4	7	11	15	20	26
1	1.2	2	8	15	16	19	20
2	2.05	1	3
3	2.83	2	3	5	7
4	4.1	3	10	16	19	22	24
5	4.45	5	14	22	23	24	25
6	4.94	3	19	25	26	26	27

In order to study the change in hydrogen-ion concentration of the external phase in contact with the emulsifying agent and oil, a series of cottonseed oil emulsions was prepared and their hydrogen-ion concentrations measured twenty-four hours after preparation. The results of these measurements are given in Table IX.

TABLE IX. *Changes in Hydrogen-Ion Concentration in the External Phase After Emulsification of Cottonseed Oil.*

No.	pH of External Phase	Emulsify- ing Agent	pH of Emulsion	Emulsify- ing Agent	pH of Emulsion
1	0.1	Acacia	0.27	Tragacanth	0.27
2	1.07	"	1.56	"	1.37
3	2.00	"	3.57	"	2.80
4	2.9	"	4.11	"	4.22
5	3.89	"	4.22	"	4.64
6	4.64	"	4.21	"	4.50
7	5.52	"	4.11	"	4.21
8	6.97	"	4.12	"	4.49
9	8.98	"	4.26	"	4.37
10	9.86	"	4.25	"	4.80
11	10.85	"	4.28	"	9.82
12	11.74	"	9.92	"	10.93
13	12.65	"	12.10	"	12.13
14	13.53	"	13.35	"	too gela- tinous to measure

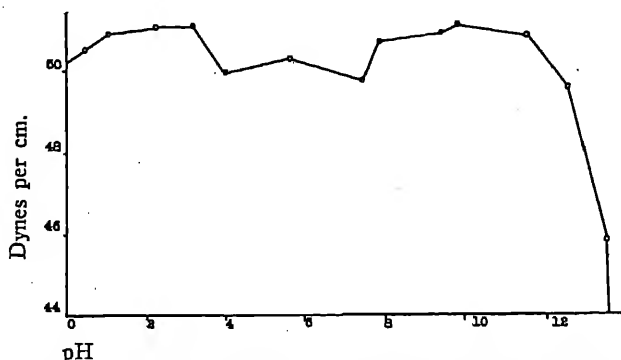
The tragacanth emulsions were diluted with an equal volume of boiled distilled water in order to sufficiently reduce the viscosity to enable the measurement of hydrogen-ion concentration to be made. The results indicate that the stable emulsions with acacia were between pH 4.11 and pH 4.28, whereas with tragacanth after making correction for

the dilution before measurement the pH of greatest stability of the finished emulsion was approximately 2.5.

These changes in hydrogen-ion concentration of the external phase after the formation of the emulsions indicate the definite buffer actions of acacia and tragacanth.

STUDY OF THE PHYSICAL PROPERTIES OF THE EMULSIONS

Surface Tension.—This property was determined at 35° C. with a standardized Du Noüy tensiometer. The results with tragacanth can be seen in Graph 5 and with acacia in Table X.



GRAPH No. 5.—Surface Tension of Emulsions with Tragacanth and Their Hydrogen-Ion Concentrations.

TABLE X. *Surface Tension of Emulsions with Acacia at 35° C.*

No.	pH	Dynes per cm.
1	0.40	54.8
2	1.00	57.3
3	2.08	62.2
4	3.02	63.0
5	4.00	64.8
6	5.60	66.1
7	7.30	66.6
8	7.75	66.2
9	9.20	65.9
10	9.95	64.1
11	11.75	62.3
12	12.25	68.1
13	13.25	59.4

Interfacial Tension.—The interfacial tension was measured at 27° C. by a pipette similar to the one used by Donnan¹⁷ and his students (see Fig. 1). The interfacial tension of water was taken as 10 and the

¹⁷ Donnan, *Z. physik. Chem.*, 31, 42 (1899) also *Brit. Med. J.*, Dec. 23, 1905.

following formula employed, where T is the interfacial tension, V the volume of oil and N the number of drops.

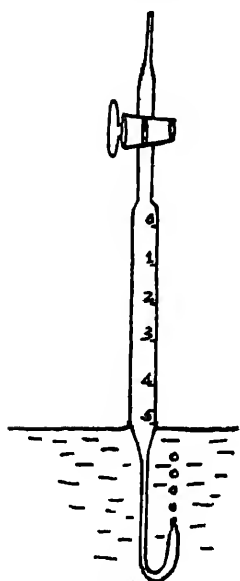


FIG. 1.—Donnan Pipette.

$$T \propto \frac{V}{N}$$

$$T = K \frac{V}{N}$$

$$\text{for pure water} \quad 10 = \frac{3K}{24}$$

$$\text{and} \quad K = 80$$

$$\text{then} \quad T = 80 \frac{V}{N}$$

The results of these measurements are plotted in Graph 6.

Viscosity.—Using distilled water as a standard at 30° C. the relative viscosities of a series of emulsions were determined by measuring the time in seconds required for a definite volume of the emulsion to run from a pipette with a capillary tip compared with the time required for the flow of the same volume of water. Through the entire range of hydrogen-ion concentration the relative viscosity of the acacia emulsions was 1.25. The viscosities of the tragacanth emulsions may be seen in Graph 7.

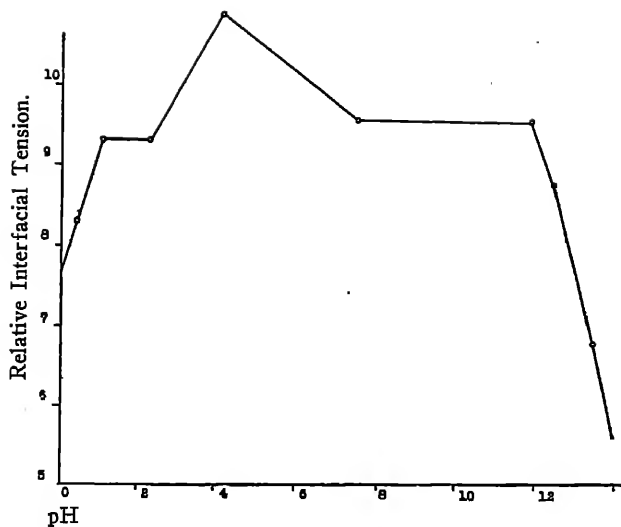
Size of Particle.—Emulsions of mineral oil were prepared after coloring the oil with alkanet root and the size of the particles measured microscopically. The results are tabulated in Tables XI and XII.

TABLE XI. *Mineral Oil Emulsified with Acacia.*

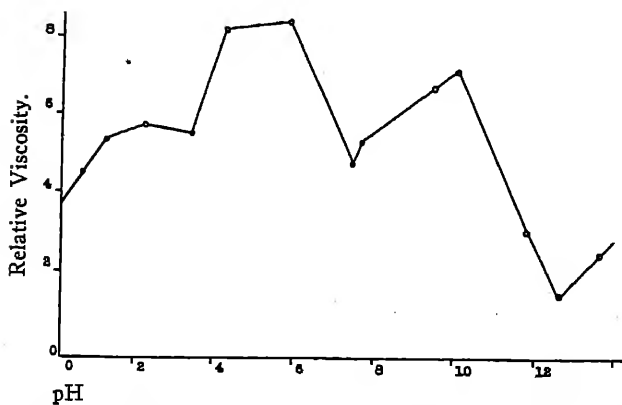
No.	pH	Average Diameter of Particles in Microns					
1	0.40	3	8	10	12	15	18
2	7.30	3	6	9	13	13	13
3	7.75	4	5	10	12	12	12
4	13.25	4	7	13	15	15	15

TABLE XII. *Mineral Oil Emulsified with Tragacanth.*

No.	pH	Average Diameter of Particles in Microns				
1	0.40	17	20	25	30	
2	1.00	17	20	25	25	
3	5.60	10	17	20	20	
4	13.25	40	50	100	110	



GRAPH No. 6.—Interfacial Tension of Mineral Oil and Solutions of Various Hydrogen-Ion Concentration.



GRAPH No. 7.—Emulsions with Tragacanth, Their Relative Viscosities and Their Hydrogen-Ion Concentrations.

Gels of tragacanth in water of different hydrogen-ion concentrations were prepared and after standing for 3 days the separation of water at the surface was observed. The gels were prepared by rubbing 0.5 grams of tragacanth with 25 cc. of water until gelatinization occurred.

METHOD OF STUDY OF THE INFLUENCE OF CHANGES OF HYDROGEN-ION CONCENTRATION UPON EMULSIONS OF THE WATER-IN-OIL TYPE

The emulsions were prepared in quantities of 40 cc. each, emulsifying in each case 10 cc. of the aqueous material, producing a 25 per cent by volume water-in-oil emulsion. Preliminary experimentation with magnesium oleate as an emulsifying agent showed that one gram of the compound was sufficient to emulsify the designated quantity of water. It was further observed that the age of the magnesium oleate influenced its emulsifying capacity. Upon keeping, even in tightly stoppered containers, the compound tends to harden and become brittle, in this condition it does not mix readily with the oil and the emulsions formed by using this substance are granular and unstable. The relative degrees of stability of the emulsions made with freshly precipitated magnesium oleate, and those made with the product which had been kept for six or eight weeks can be observed by studying the following tables of stability.

The aqueous solutions of various hydrogen-ion concentrations were prepared as in the foregoing experiments by the addition of various quantities of hydrochloric acid and sodium hydroxide to distilled water. The pH of these solutions was determined electrometrically. As these unbuffered mixtures, near the neutral point, change in hydrogen-ion concentration quite quickly, the emulsions of the mixtures near pH 7 were prepared as soon as possible after the hydrogen-ion concentration measurement.*

The emulsification was accomplished by triturating the emulsifying agent with 18 cc. of oil at 25° C. and adding, in one portion, the 10 cc. of aqueous fluid. After brisk trituration the formation of the emulsion nucleus could be easily ascertained by the sudden increase in viscosity and the occurrence of a crackling sound when the pestle was pulled through the emulsion. With continued trituration the emulsion nucleus was diluted to the proper volume and stored in dry, well-stoppered graduated containers.

The separation of inverted emulsions must not be mistaken for the gradual settling of the heavier emulsified water to the bottom of the container, for this might easily be incorporated by agitation. When actual separation occurs there appears a clear aqueous fluid at the

* The pH measurements indicate the pH of the internal phase prior to mixing.

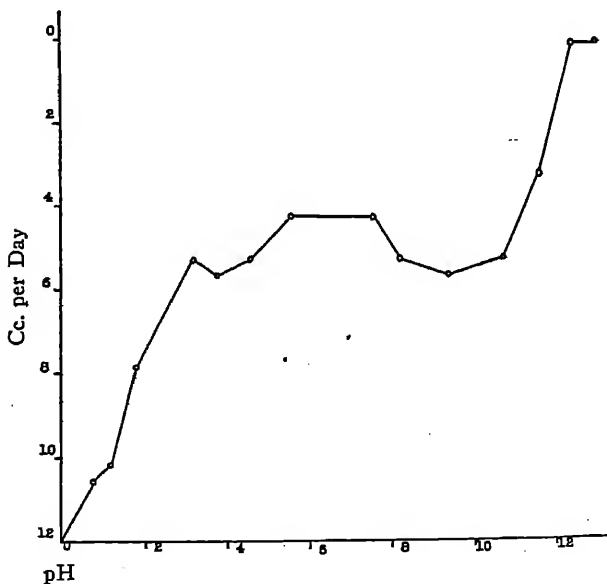
TABLE XIII. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.*
Degree of separation in cc. in different time periods.

No.	pH	Hours		Days													
		½	1	1	3	4	7	11	15	20	28	38	45	52	59	66	73
1	0.9	10	c.s.	9	9	9	9	9	9	9	9	9	9	9	9	9	9
2	1.36	9	9	6	7	8	8	8	8	8	8	8	8	8	8	8	8
3	1.87	2	2	3	4	5	5	5	5	5	5	5	5	5
4	3.05	2	3	4	4	4	4	4	4	4	4	4	4	4
5	3.97	1	2	2	2	2	3	3	3	3	3	3	3
6	4.85	1	2	2	2	3	3	3	3	3	3	3
7	5.55	1	2	2	2	3	3	3	3	3	3	3
8	7.55	1	2	2	2	3	3	3	3	3	3	3
9	8.02	2	2	3	4	4	4	4	5	5	5	5	5	5
10	9.25	2	2	3	3	4	4	4	5	5	5	5	5	5
11	10.97	3	3	4	5	5	5	5	5	5	5	5	5	5
12	11.75	1	1	1	1	2	2	2	2	2	2
13	12.38
14	13.0	yel- low	gran- ular

c.s. = complete separation.

bottom of the emulsion which cannot be reincorporated by agitation. With certain emulsions of this type, employing freshly precipitated magnesium oleate, the authors have observed no separation in a period of eight or ten months. When, however, emulsions are prepared with older samples of the emulsifying agent, the separation begins in a much shorter period of time.

The following observations were made upon the effect of changes of pH in water-in-oil emulsions.



GRAPH NO. 8.—Stability of Emulsions in Mineral Oil and Their Hydrogen-Ion Concentrations.

The ordinates of this graph represent the amount of separation per day over a period of ninety-four days and multiplied by ten to eliminate fractions.

Table XIII indicates that the stable point of the internal phase of these emulsions is near the point, pH 12.38. The magnesium oleate employed in these emulsions had been prepared about fourteen days prior to its use. Graph 8 shows the average separation of a series of emulsions over a period of ninety-four days.

Figure 2 shows the emulsions from Table XIII after thirty days.

Reading from left to right these emulsions are numbered as given in Table XIII.

With magnesium oleate which was freshly precipitated, as observed with the foregoing experiments, the range of hydrogen-ion concentrations at which the emulsions are unstable is on the acid side. With

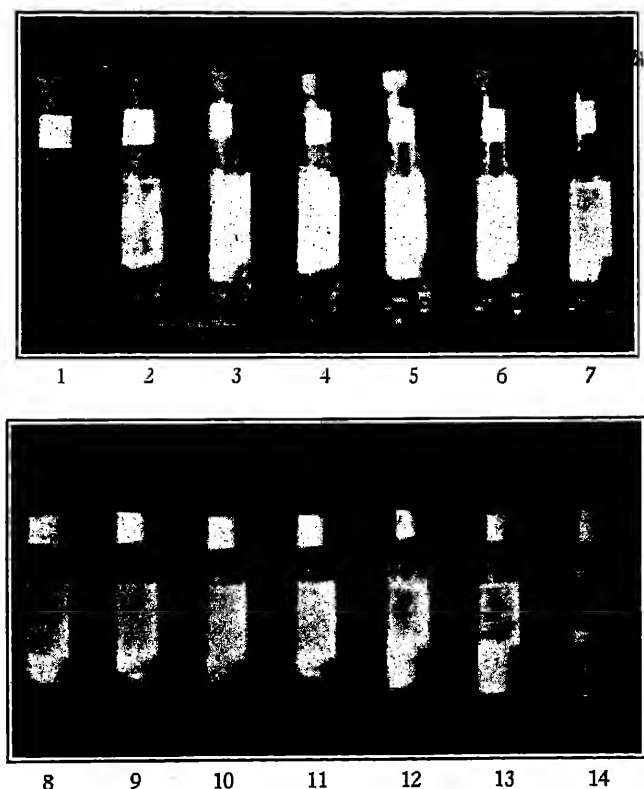


FIG. 2.—Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.

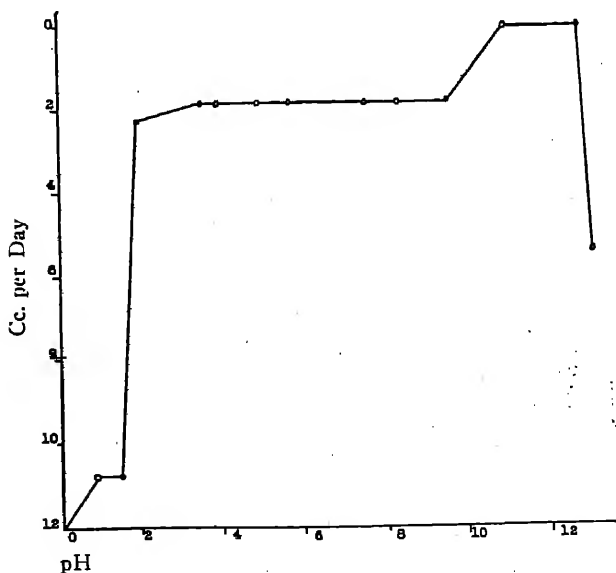
the freshly precipitated emulsifying agent practically the entire range of the pH scale from pH 1.87 to pH 12.38 were stable as shown by Table XIV. Yet on the alkaline side of the pH scale, pH 10.97, 11.75 and 12.38, the degree of creaming was less than when the internal phase was closer to the neutral point. Table XIV shows the stability of these emulsions over a period of eighty-six days.

TABLE XIV. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil. (Freshly Precipitated Magnesium Oleate.)*

Degree of separation in cc. in different time periods.

No.	pH	Hours					Days									
		1	3	7	12	20	30	37	44	51	58	65	72	79	86	
1	0.9	8	8	9	9	9	9	9	9	9	9	9	9	9	9	
2	1.36	..	1	1	1	1	1	1	1	1	1	1	1	1	1	
14	13.0	Coarse solid mass														

When olive oil was employed as the external phase, using the same magnesium oleate as in the first series of emulsions, the emulsions as a whole were more permanent. The degree of separation of this series of emulsions are given in Table XV and the summary of a series of these results is plotted in Graph 9.



GRAPH No. 9.—Stability of Emulsions in Olive Oil and Their Hydrogen-Ion Concentrations.

The ordinates of this graph represent the amount of separation per day over a period of ninety-one days and multiplied by ten to eliminate fractions.

TABLE XV. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Olive Oil.*

Degree of separation in cc. in different time periods.

No.	pH	Hours							Days										
		½	1	1	4	12	17	25	35	42	49	56	63	70	77	84	91		
1	0.9	10	c.s.		
2	1.36	..	8	10	c.s.		
3	1.87	1	2	2	2	2	2	2	2	2	2	2	2	2	2		
4	3.05	1	1	1	1	1	1	1	1	1		
5	3.97	1	1	1	1	1	1	1	1		
6	4.85	1	1	1	1	1	1	1	1		
7	5.55	1	1	1	1	1	1	1	1		
8	7.55	1	1	1	1	1	1	1	1		
9	8.02	1	1	1	1	1	1	1	1		
10	9.25	1	1	1	1	1	1	1	1		
14	13.0	yel-low	yel-low	1	6	6	6	6	6	6	6	6	6		

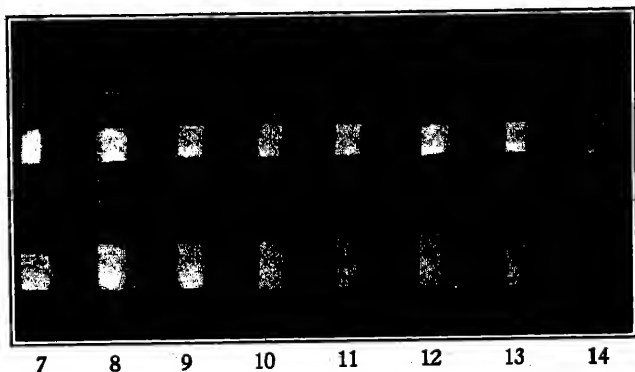
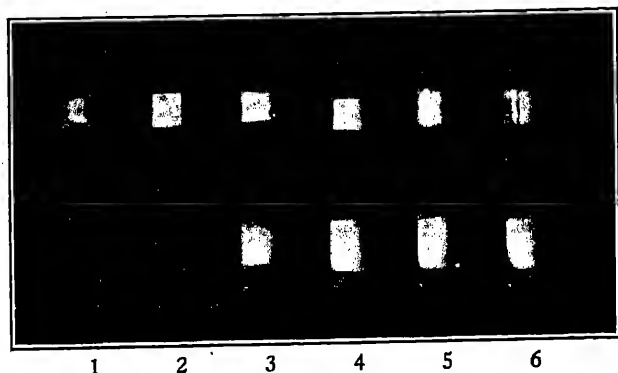


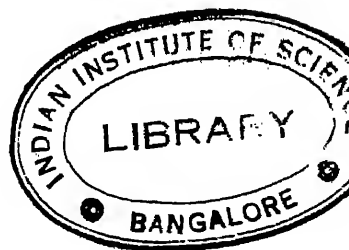
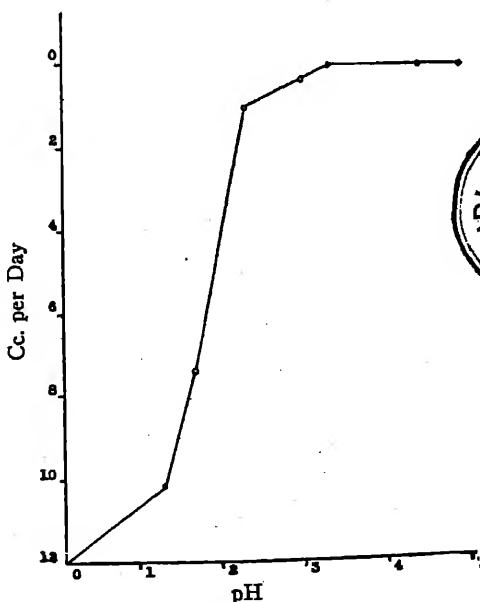
FIG. 3.—Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Olive Oil.

TABLE XVII. *Emulsions of Solution of Sodium Chloride in Olive Oil.*

Degree of separation in cc. in different time periods.

No.	Nor- mality	Days										
		1	4	9	14	22	32	39	46	53	60	67
1	1	1	3	3	3	3	3	3	3	3	3	3
2	10^{-1}	1	2	2	2	2	2	2	2	2	2	2
3	10^{-2}	1	1	1	1	1	1	1	1	1	1	1
4	10^{-3}	1	1	1	1	1	1	1	1	1	1	1

In order to study the influence of hydrogen-ions produced by a source other than hydrochloric acid, solutions of sulfuric acid were prepared and their pH determined. These were then emulsified in mineral oil and olive oil. With olive oil and mineral oil the results were similar to those observed when hydrochloric acid solutions were emulsified. Tables XVIII and XIX record these results.



GRAPH No. 10.—Stability of Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil (H_2SO_4).

Graph 10 shows the stability of the sulfuric acid solutions emulsified in mineral oil.

TABLE XVIII. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.*

Degree of separation in cc. in different time periods.

No.	pH	Hours						Days									
		1	2	1	4	9	14	22	32	39	46	53	60	67	74	81	88
1	1.04	10	c.s.
2	1.31	4	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
3	2.17	1	1	1	1	1	1	1	1	1	1	1	1	1
4	3.06	no separation		
5	4.21	"	"	
6	4.86	"	"	

c.s. = complete separation.

TABLE XIX. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Olive Oil.*

Degree of separation in cc. in different time periods.

No.	pH	Hours						Days									
		½	1	4	9	17	27	34	41	48	55	67	69	76	83		
1	1.04	8	8	8	8	9	9	10	c.s.
2	1.51	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
3	2.17	..	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3
4	3.06	..	2	2	3	3	3	3	3	3	3	3	3	3	3	3	3
5	4.21	..	1	1	1	1	2	2	2	2	2	2	2	2	2	2	2
6	4.86	..	1	1	1	1	1	1	1	2	2	2	2	2	2	2	2

c.s. = complete separation.

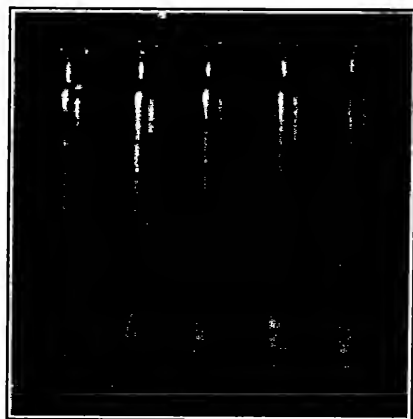


FIG. 5.—Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.

In order to determine within a closer range the hydrogen concentration at which the range of instability on the acid side begins the

following series of emulsions were prepared using mineral oil as the external phase. Table XX shows the degree of separation of these emulsions over a period of eighty-three days, and Figure 5 shows the emulsions listed in Table XX after thirty days. Reading from left to right the emulsions in Figure 5 are numbered as given in Table XX.

TABLE XX. *Emulsions of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.*

Degree of separation in cc. in different time periods.

No.	pH	Hours			Days									
		½	4	9	17	27	34	41	48	55	62	69	76	83
1	0.9	5	10	c.s.
2	1.36	2.5	3	3	3	3	3	3	3	3	3	3	3	3
3	1.87	1	1	1	1	1	1	1	1	1	1
4	2.17	1	1	1	1	1	1
5	3.05

c.s. = complete separation.

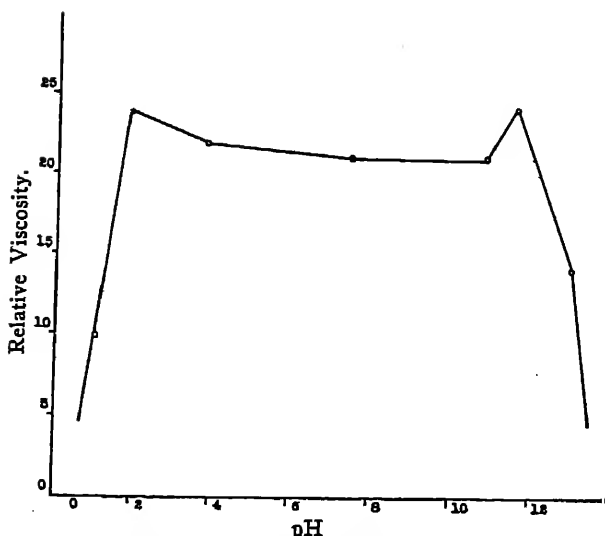
STUDY OF THE PHYSICAL PROPERTIES OF EMULSIONS OF THE WATER-IN-OIL TYPE

Surface Tension.—The surface tensions of the various emulsions of solutions of different hydrogen-ion concentrations in mineral oil were measured at 20° C. with a standardized Du Noüy tensiometer. The results of these measurements are tabulated in Table XXI.

TABLE XXI. *Surface Tension of Emulsions in Mineral Oil at 20° C.*

No.	pH	Dynes per cm.
1	Mineral Oil	35.62
2	0.9	36.33
3	1.87	37.08
4	3.97	36.81
5	7.55	36.81
6	9.25	36.43
7	10.97	36.60
8	11.75	37.08
9	13.0	36.64

Viscosity.—Using the external phase, mineral oil, as a standard at 20° C, the relative viscosities of a series of emulsions were determined by the method previously described. The relative viscosities of these emulsions showed little variation except on the extreme acid and alkaline sides of the pH range. The results of these measurements are tabulated in Table XXII and plotted in Graph 11.



GRAPH No. 11.—Relative Viscosity of Solutions of Various Hydrogen-Ion Concentrations in Mineral Oil.

TABLE XXII. *Relative Viscosities of Emulsions in Mineral Oil at 20° C.*

No.	pH	Relative Viscosities
1	Mineral Oil	243 seconds unity
2	0.9	0.106
3	1.87	0.242
4	3.97	0.224
5	7.55	0.219
6	9.25	0.214
7	10.97	0.214
8	11.75	0.245
9	13.0	0.144

Size of Particle.—Emulsions of solutions of various hydrogen-ion concentrations (colored with a water-soluble dye) were prepared using mineral oil as the external phase. The size of the particles was measured microscopically. The results of these measurements are given in Table XXIII.

TABLE XXIII. *Size of Particles of Emulsions in Mineral Oil.*

No.	pH	Average Diameter of Particles in Microns
1	0.9	38.9
2	1.87	21.2
3	3.97	20.6
4	7.55	17.7
5	9.25	22.4
6	10.97	17.7
7	11.75	30.0
8	13.0	30.0

SUMMARY OF RESULTS AND THEORETICAL CONSIDERATIONS

EMULSIFYING AGENTS

With mineral oil, as with Harkins' observation with benzene magnesium, calcium, cobaltous, nickelous, manganous and aluminum oleates produce emulsions of the water-in-oil type. With valeric acid, as with Harkins' and Newman's observations with oleic acid, the univalent salts produce oil-in-water emulsions, whereas the salts of divalent metals produce water-in-oil emulsions. The salts of arabic acid produce oil-in-water emulsions irrespective of the valence of the metallic atom in combination. It is entirely possible that acacia always tends to produce an oil-in-water emulsion because of the buffered emulsion characteristic which has been demonstrated, or that it is the divalent salt of an acid in which there are several hydroxyl groups. According to the observations made with the divalent salts of *i*-galactonic and gluconic acids it is possible that the presence of hydroxyl groups in the molecule of arabic acid in addition to the fact that acacia contains some univalent salts of arabic acid, is partly responsible for its invariably producing emulsions of the oil-in-water type. As the gum tragacanth is essentially the calcium salt of bassoric acid and produces emulsions of the oil-in-water type, it is probable that this condition exists for reasons similar to those proposed for the salts of arabic acid.

It is interesting to note that Weston¹⁸ working with colloidal clays, observed that these substances would produce emulsions of the oil-in-water or water-in-oil type.

INFLUENCE OF CHANGES IN pH UPON OIL-IN-WATER EMULSIONS

The data and graphs correlating the results obtained indicate that vegetable and mineral oil emulsions prepared with acacia are stable with a hydrogen-ion concentration of the external phase varied from pH 2 to pH 10. The presence of alkalies is especially detrimental to the stability of these emulsions. Emulsions with acacia at various hydrogen-ion concentrations show little change in their surface tensions and their relative viscosities. The size of the particles in the acacia emulsions are far more uniform and smaller than those of the tragacanth emulsions and on the acid and alkaline sides of the pH scale there is a slight increase in the size of the particle. This is exactly what one would expect as at these points also the smallest degree of stability was observed. Microscopically it was observed that this increase in the size of the particle is due to coalescence, preliminary to separation.

The emulsions prepared with tragacanth are especially stable when the external phase is adjusted at pH 1.9 to pH 2.3 and quickly separate

¹⁸ Weston, *Chem. Age (London)*, 4, 640 (1921).

on the alkaline side of the pH scale. Examination of Graph 4 indicates that this range of hydrogen-ion concentration does not change when the acidity is produced by sulfuric acid instead of hydrochloric acid. We cannot, however, consider this as a specific effect of the hydrogen ion alone for sodium chloride produces a similar stability when the sodium-ion concentration is about $10^{-2} N$.

A study of Graph 7 indicates that there is a considerable drop in viscosity with an increase of hydroxyl-ion concentration, which is characteristic of most mucilaginous material. In other words the presence of alkali reduces the viscosity of the gel. There was no change in viscosity in the emulsions prepared with acacia.

The authors feel that it is not a maximum viscosity which is desirable as indicated by Graph 7, but an optimum relative viscosity which is found to be between 4 and 6. Holmes and Child¹⁹ working with gelatin solutions support this view. It seems that in tragacanth emulsions, as observed by other investigators in other fields, viscosity aids emulsification solely by virtue of the hindrance offered to agglutination of the oil particles.

Graph 5 shows that those emulsions prepared with tragacanth decrease in surface tension toward the alkaline side of the pH scale. The authors, however, do not consider the phenomenon of surface tension of paramount importance in view of the postulates of Langmuir²⁰ who states that similar liquids may have the same surface tension against air, owing to the fact that in their surface layer similar groups or atoms may be similarly oriented.

The interfacial tension of the two liquids has been used by some as a measure of the emulsifying power of one liquid upon another. Accordingly this measurement was attempted between mineral oil and tragacanth gels of various hydrogen-ion concentrations. Invariably a steady stream was obtained instead of drops as was obtained by Donnan²¹ with solutions of the sodium salts of certain high molecular weight fatty acids. The interfacial tension of mineral oil and solutions of various hydrogen-ion concentrations as plotted in Graph 6 show that the alkaline solutions reduce the interfacial tension which should increase the power of emulsification, were the emulsifying agent not affected. It is concluded therefore that the changes in hydrogen-ion concentration influence the permanency of the gel and thus affect the stability of the emulsion.

Gels prepared with tragacanth showed that only those gels between pH 0.4 and 2.1 remain free from the separation of water at the surface, or in other words the liquids between pH 1 and 2.1 prepare the most stable gels with tragacanth; it will be recalled that the stable

¹⁹ Holmes and Child, *J. Am. Chem. Soc.*, 42, 2049 (1920).

²⁰ Langmuir, *J. Am. Chem. Soc.*, 39, 1843 (1917).

²¹ Donnan, *Kolloid-Z.*, 4, 208 (1910).

range of pH for emulsions made with tragacanth practically lies within this scale. This supports Fischer's²² hydrate theory of emulsification, which postulates that oil is most permanently emulsified in a hydrophile colloid when just a sufficient amount of water is present to form a hydrate. With tragacanth the amount of water taken up seems to be a function of its hydrogen-ion concentration. At the range of the pH scale where tragacanth shows itself to possess the highest degree of hydratability, this range is the most stable point for emulsions prepared with this colloid.

INFLUENCES OF CHANGES IN pH UPON WATER-IN-OIL EMULSIONS

A study of the graphs showing the stability of the emulsions in mineral oil and olive oil (graphs 8 and 9) indicate that the most stable range of hydrogen-ion concentration for the internal phase of these emulsions lies well on the alkaline side of the pH scale. The pH range at which the emulsions were most stable was between 11 and 12.5. From pH 11 to pH 2.5 there is a range of moderate stability, whereas the region from pH 2.5 to pH 0.9 may be looked upon as the range of extreme instability. With freshly precipitated magnesium oleate there is little or no separation from pH 2.5 to 11 but the field of instability is on the acid side of pH 2.5 and the maximum point of stability lies between pH 11 and 12.5.

With mineral oil and olive oil the similar observations were made if the hydrogen-ion concentration was produced by the addition of sulfuric acid indicating that the instability was due to the presence of a high hydrogen-ion concentration. When various concentrations of solutions of sodium chloride were emulsified, there was no influence in stability as far as emulsions in mineral oil were concerned, but with those dispersed in olive oil the instability of the emulsions increased with the increase of concentration of sodium chloride in the dispersed phase.

A study of the physical properties of the emulsions shows that as far as surface tension is concerned (Table XXI) there is no significant difference in any of the emulsions. Viscosity measurements show (Table XXII) that the emulsions of liquids of various hydrogen-ion concentrations in mineral oil are far less viscous than the external phase alone. On the extreme acid and alkaline sides of the pH scale (pH 0.9 and pH 13) there was a marked drop in viscosity. It is of interest to note that these emulsions of extremely low viscosity were those which were least stable.

The size of the particles of the emulsions in mineral oil increased on the alkaline side of the pH scale without any appreciable influence

²² M. Fischer and M. Hooker, "Fats and Fatty Degeneration," New York, Wiley & Sons, Inc., 1917, p. 5.

upon stability. The unstable emulsion of an internal phase of pH 0.9 had particles which were larger than those in any of the other emulsions. A careful microscopic study of the size of the particles in relation to stability showed that in emulsions, when the average size of the particles was between 17 and 30 microns there was no influence on the stability. Particles above 30 microns (average size) tend to coalesce and separate as a layer beneath.

CONCLUSIONS

1. The character of emulsions produced by several oleates, certain univalent and divalent salts of arabic acid and some related compounds has been studied.

2. The range of greatest stability for either vegetable or mineral oil emulsions prepared with acacia is attained when the pH of the outer phase lies between pH 2 and 10 and with tragacanth the range is from pH 1.9 to 2.3. The stable pH range of the acacia emulsions lies between pH 4.11 and 4.28, and with tragacanth the stable point is approximately pH 2.5. See Table IX.

3. The size of the particles, surface tension, interfacial tension and viscosity have been determined at various points on the pH scale. Changes in particle size and viscosity are caused by changing the hydrogen-ion concentration of emulsions prepared with tragacanth. The viscosities of acacia emulsions are not altered by changing the pH, the size of the particle, however, increases on the alkaline side of the pH scale.

4. There are indications of Fischer's hydrate theory being substantiated in emulsions prepared with tragacanth.

5. With water-in-oil emulsions prepared with magnesium oleate, olive oil is found to be a more stable dispersion phase than mineral oil. The most stable range of hydrogen-ion concentration of the internal phase was found to be between pH 11 and 12.5. With a hydrogen-ion concentration more acidic than pH 2.5, extreme instability was observed.

6. With emulsions of the water-in-oil type changes in pH of the inner phase did not affect the surface tension, the unstable emulsions had a lower viscosity than the stable ones and the size of the particles does not alter the stability up to 30 microns average size.

University of Maryland,

College Park, Md.

and

Pharmaceutical Research Laboratory,

Sharp & Dohme,

Baltimore, Md.

NEW MICROSCOPIC METHODS IN CONNECTION WITH THE PROBLEM OF VULCANIZATION

By E. A. HAUSER in collaboration with H. MIEDEL and
M. HÜNEMÖRDER

It is nearly 100 years ago since the German chemist Lüdersdorff noticed that rubber dissolved in turpentine containing sulfur and heated in the state of solution will give, after evaporation, a rubber film showing decreased tackiness on its surface. The observations of Goodyear in America and, shortly afterwards, the independent experiments by Hancock in England marked the discovery of the process of treating rubber with sulfur which lies at the basis of the modern rubber industry. It is today a well-known fact that crude rubber immersed into molten sulfur, or thoroughly mixed with sulfur and afterwards exposed to heat, will undergo very marked changes, for example becoming more or less temperature-resistant. It furthermore loses the tacky feeling peculiar to crude rubber and regains its elastic properties, which have been destroyed in the kneading process called mastication. This fundamental discovery has been termed "vulcanization."

The first really systematic work directed toward a scientific interpretation of this phenomenon has, however, been carried out only during the last few years and credit is herewith given to C. O. Weber,¹ who, I believe, is entitled to be called the father of modern rubber chemistry. Although progress in the science has gone beyond some of his assumptions, he must nevertheless be credited with having been the first to attempt to get a deeper insight into the problem of vulcanization by microscopical observations. The first microscopical experiments in regard to the so-called blooming of sulfur in vulcanized rubber goods are due to him.² A short time afterwards the Frenchman Breuil published a masterly paper on the microscopy of the process of vulcanization.³ This paper is unique in the amount and correctness of the observations mentioned therein, especially when we consider that the microscopical technique at that time was far less developed than it is today. It is a great pity, therefore, that this work has remained prac-

¹ See Weber's "The Chemistry of India Rubber" (Fifth Impression), London, 1919, p. 41 *et seq.*

² *Op. cit.*, p. 110 *et seq.*

³ *Caoutchouc et gutta-percha*, 2, 82-83, 118-23, 158-61, 197-205 (1905).

tically unknown, as, in my opinion, it must be regarded as the basis for all future work along these lines.

A further contribution is due to Loewen,⁴ who, although working in principle according to Breuil's method, obtained more valuable results on account of the fact that he greatly improved the working method. Finally, reference should be made to a paper by Regnaud,⁵ who studied the blooming procedure microscopically and drew certain conclusions in regard to the vulcanization from his observations. In all the work mentioned, however, the actual vulcanization had to be carried out independent of the microscopical observation, as no means were available at the time to cure the samples under the microscope. All observations therefore refer to a short time after termination of the heating period. In recent years Dannenberg published some research work,⁶ carried out in my laboratory, which differs in principle from the aforementioned methods, as he managed to observe the whole process of vulcanization under the microscope. The instrument used for this purpose is an electrically heated "squeezing chamber," which can be placed on the table of the microscope. This method possesses the advantage that a continuous observation is possible. There are, however, certain disadvantages connected with the method. The construction of the heating chamber could not avoid a considerable drop in temperature between the place where the temperature of the chamber can be measured and the actual position of the sample. In Dannenberg's publication this fact has not been sufficiently considered with the result that some observations, especially the formation of colloidal sulfur during the melting period, have in the meantime been proved to be incorrect. A further, although not so important, drawback is the fact that no exact comparison with large-scale vulcanization was obtainable, as only very small vulcanizing units are fitted with electrical heating. From the aforementioned it is easy to realize the importance of devising a method, which would not only allow one to draw an absolute comparison with practical equipments, but which would also be so simple and easy in its handling, that it would enable one to investigate quickly the various problems in connection with vulcanization which crop up daily in normal rubber manufacture. A marked step toward the solution of the latter part of the problem has undoubtedly been made by Grenquist, who has carried out extensive experiments on the microscopical observation of pigment dispersion during vulcanization in the research laboratories of the Fisk Rubber Company.⁷

⁴ *Gummi-Ztg.*, 27, No. 32.

⁵ *Chim. Ind.*, 18, 93 (1927).

⁶ *Kautschuk*, 3, 104, 128 (1927).

⁷ Paper delivered at the St. Louis meeting of the American Chemical Society, April, 1928.

I have now devised two microscopical attachments which enable the continuous observation of the process of vulcanization in direct comparison with the normal steam autoclave, or with the vulcanization of molded goods in so-called steam-heated platen presses (Fig. 1).

Although we must admit that we are today in the very infancy of this line of research and therefore are not yet in a position to make

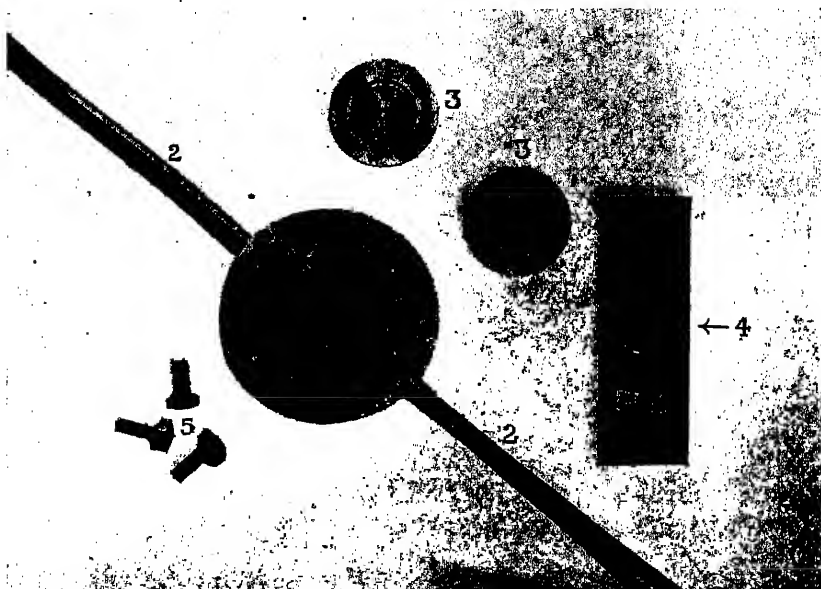


Fig. 1.

1—Hollow steel plates.
2—Steam inlet and outlet pipes.
3—Vulcanizing mould.

4—Sample of rubber compound placed between two coverplates to be introduced into the mould.
5—Screws for tightening the plates.

definite statements in regard to the actual cause of vulcanization, I believe that the results obtained with the method referred to are already of sufficient interest to demonstrate the scientific and practical value of such a field of research.

For the sake of simplicity we will consider only three systems according to the following formulas:

100 parts rubber
3 " sulfur

100 parts rubber
3 " sulfur
5 " zinc oxide

100 parts rubber
3 " sulfur
5 " zinc oxide
1 " accelerator

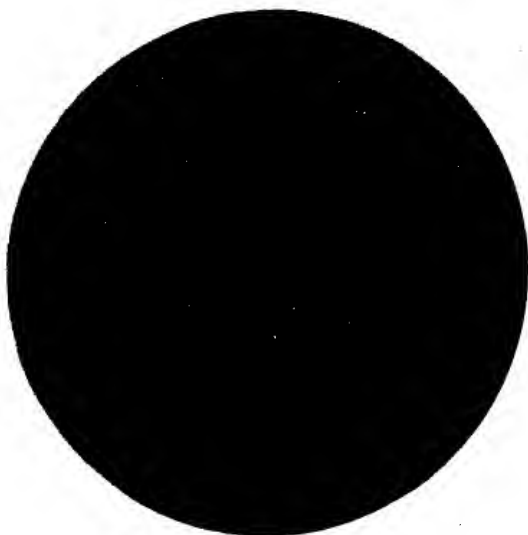


FIG. 2.

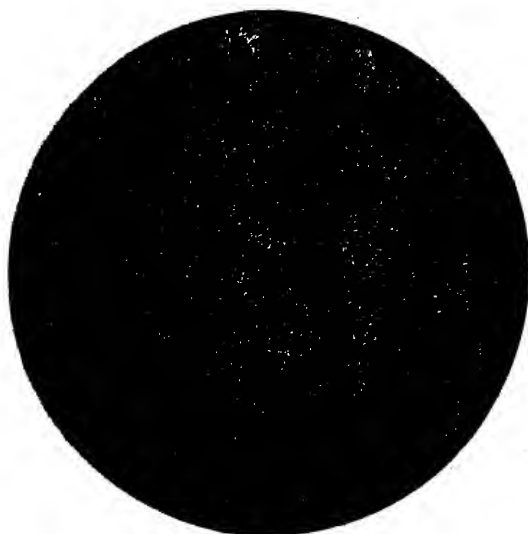


FIG. 3.

A systematic investigation shows in case 1 that whenever the mix is still in a state of undercure, the sulfur will, after termination of the heating period, reappear more or less quickly in the form of globules of colloidal dimension (Fig. 2), which increase in size, and in time link up into chains, out of which lateral crystals of various shapes begin to grow. The second case differs only in so far as the reappearance of sulfur is retarded, presumably due to a chemical reaction taking place between the sulfur and the zinc oxide.⁸ This last assumption is strengthened by the fact that the visible zinc oxide particles show an increasing discoloration or darkening during the process of vulcanization and, furthermore, that the speed with which the sulfur reappears is inversely proportional to the fineness, or, in other words, to the exposed zinc oxide surface in a unit volume. The reappearance of the sulfur in the case of zinc oxide compounds is, in general, finer, and also the formation of crystals tends to far more subdivided forms (Fig. 3). I believe that Grenquist is absolutely correct in his assumption that the crystal growth is directed in the line of least resistance, and, therefore, these forms must naturally be largely subdivided in the presence of a dispersion like zinc oxide. In the third case we notice that no sulfur reappears in the above-described forms and only after a few days can we detect on the surface of the sample the formation of rhombic sulfur crystals (Fig. 4). This observation seems to be of general practical importance, in that we can easily make use of this fact to determine in a very simple and rapid manner whether a given substance will show accelerating properties or not. The formation of surface crystals also takes place in cases 1 and 2. After a few days the formed crystal structure in the interior of the compound begins to fade away and, at the same time, surface crystallization can be observed. I believe, therefore, that these observations must lead us to distinguish sharply between the so-called "bloom-ing," which is undoubtedly a surface effect, and the effect of rubber compounds which have become transparent immediately after vulcanization, but which gradually become more and more opaque due to the reappearance of sulfur throughout the compound.

Of all the accelerators investigated, thiuram disulfide has shown an absolutely individual behavior. When the vulcanized compound containing thiuram is allowed to cool down, the formation of extremely fine needle-shaped deposits can be observed, which in time agglomerate to form half-moon-like crystals (Fig. 5). It has not been possible as yet to determine with certainty whether these formations are a special kind of sulfur recrystallization or whether they are partly due to a recrystallization of the accelerator.

There is another important difference between the three groups

⁸ See Pohle, *Z. wiss. Mikroskop.*, 44, 183 (1927).



FIG. 4.



FIG. 5.

mentioned above in that the pure rubber sulfur compound and the compound containing zinc oxide show a marked flow at the beginning of vulcanization, whereas the flow in the case of accelerator mixes always will be very limited and will stop quite suddenly. The flow can very well be observed in mixes containing zinc oxide or other pigments and, furthermore, we can observe very clearly changes in the degree of dispersion taking place. In this connection it should be mentioned that the various pigments behave very differently in this respect, some tending to attain a high degree of dispersion, whereas others will tend to agglomerate.⁹

In the further course of investigations which are published more in detail in *Le caoutchouc et la guttapercha*, much time was devoted to the study of vulcanizing properties of the various fractions of rubber hydrocarbons obtainable by the method described by Pummerer.¹⁰ Also in this respect we have for the sake of simplicity carried out all the important observations on a compound 100 parts of rubber and 3 parts of sulfur. In close analogy to other physical data obtained with the various fractions, a distinct difference in the speed of flow can be observed. The α rubber hydrocarbon, which today may be regarded with certainty as a hydrocarbon of low agglomeration and readily soluble in ether, will show the strongest flow and will continue for the longest time, whereas the β hydrocarbon, the highly agglomerated insoluble fraction, will show such a slight flow that even the addition of accelerators will not cause marked differences, while the addition of accelerators to the α hydrocarbon produces a spontaneous solidification. A further difference is the ease with which sulfur dissolves in the two fractions. The dissolution in α rubber is slower and requires higher temperatures than the total hydrocarbon or the β fraction. If we follow up the vulcanization of so-called total rubber hydrocarbon microscopically, we find that in all cases where the time of cure would suffice to obtain a properly vulcanized product the sulfur reappears at a few points of the sample after some minutes of cooling. It is known that by submitting total rubber hydrocarbon to ether diffusion two fractions can be obtained, which are termed α rubber in case of the easily soluble and β rubber for the practically insoluble fraction. It has been ascertained that when carrying out the diffusion with all necessary precautions and when using a thoroughly prepared acetone-extracted total rubber hydrocarbon, the second obtainable α fraction will expel the sulfur immediately after vulcanization, even if this vulcanization has been carried on for hours. The same result can be obtained with the β fraction, if we use an absolutely fresh material and especially a ma-

⁹ See Grenquist, *op. cit.*

¹⁰ *Kautschuk*, April, 1926.

terial which has been thoroughly freed from any trace of α rubber still present. This fact seems important as the first α fraction obtainable does not eliminate the sulfur even if the time of heating is reduced to a couple of minutes. If we submit such a fraction again to an acetone extraction we obtain an oily residue, which has not yet been chemically identified. We believe, however, that it is an auto-oxidation product, since we have been able to reproduce the same effect by oxidizing a chemically pure α rubber. Fractions which have been extracted as mentioned will eliminate the sulfur in the same way as above described. If the extract is newly added to the compound the elimination is completely stopped. Although it may be difficult to give a comprehensive explanation of this most peculiar effect at the present moment, I believe that we can be certain that vulcanization or, in other words, the retention of the incorporated sulfur, depends on minute traces of a substance present in crude rubber, a substance which firmly adheres to the α fraction, so that it can be removed only after this fraction has been isolated from its original combination in the rubber.

SUMMARY

An attempt has been made to outline a very special line of modern rubber chemistry and to show a new method of attack. The importance is stressed of applying colloid chemical methods, when it is the aim to trace minute amounts of substances in such complicated bodies as the natural colloids and to bring these substances into our hands, so that they can then be further identified and studied by the classical methods of organic chemistry.

*Frankfurt am Main,
Germany.*

PREPARATION AND PROPERTIES OF AQUEOUS RUBBER DISPERSIONS

By H. L. TRUMBULL

*"No means which have yet been discovered seem competent when the caoutchouc has once been aggregated to restore it to its pristine state."*¹

INTRODUCTORY

The shipment of latex from Tampico to England in "good sound barrels" was attempted by Thomas Hancock² in 1830, but much to his dismay upon opening the barrels he found a solid mass of rubber and a brown fluid. Some twenty years later he demonstrated that it was possible to preserve latex by treatment with ammonia so that it would withstand shipment, but commented rather pessimistically concerning the commercial utilization of latex on account of the cost of containers and the freight rate for transportation of water. Strangely enough his views have prevailed until within the last decade when large-scale operations in Europe and America have demonstrated that the shipment of latex presents no insurmountable difficulty. It would seem, however, that the adverse experience of Hancock served to delay for nearly a century the attempt to use latex in manufacturing operations.

PREPARATION OF RUBBER DISPERSIONS

The problem of producing a rubber latex from crude rubber was assigned by Dr. W. C. Geer of the Goodrich Laboratories first to Dr. John B. Dickson and subsequently to the writer. Both men first approached the problem from the classical point of view, which is to disperse a rubber cement in an aqueous medium containing protective colloids.³ Although dispersions were obtained such as those which had been previously prepared by the workers in this field, it was obvious that these dispersions did not resemble latex for at least three reasons, firstly, the concentration of rubber was low, not over 5 to 10 per cent; secondly, solvent invariably remained in the rubber particles, hence the

¹ M. Faraday, *Quart. J. Sci. Arts*, 21, No. 41 (1826). See Hancock, "Personal Narrative" (1857), p. 19.

² "Personal Narrative" (1857), p. 29.

³ Alexander, *Chem. Ztg.*, 47, 897 (1923).

dispersed phase was a rubber cement rather than rubber; thirdly, the particles formed under any set of circumstances were larger and creamed much more rapidly than latex particles.

It occurred to the author that if rubber could be frozen in order to embrittle it, the action of a high-speed abrasive wheel on its surface might be effective in producing a dispersion. A block of masticated rubber was first allowed to absorb about an equal volume of benzene and then frozen for several days at -39° C. Immediately upon removal from the refrigerator the solid block was held closely against a high speed aloxite wheel which was porous enough to permit a lubricant of five per cent glue in water at 0° C. to flow through it from the axis to the periphery. This lubricant served as a protective colloid for the dispersion. Under the vigorous abrasive action rubber particles were sheared off the face of the block, and some of them were small enough to remain suspended in water over night (less than 1 % concentration).

The bulk of the rubber was either not removed from the block or was rolled off to form pea-sized pellets. Heat, developed by friction, apparently softened the rubber at the contact point so that it could not be abraded as could a brittle solid. Nevertheless the fact remained that some rubber had been dispersed, and that by a mechanical method.

An operable method of effecting rubber dispersion was arrived at as a result of the joint invention of Dr. J. B. Dickson and the author.⁴ This method required two smooth-roll rubber mills, one to masticate rubber and the other to serve as the dispersing machine. In order to operate the latter a thick paste of equal parts of glue and water was placed upon the rolls of one mill, and rubber from the other mill was cut in thin strips and gradually worked into the glue paste preferably at a temperature of about 70° C.

Gradual disintegration of the rubber was effected in this manner, so that further additions of rubber strips could be made until at the end of about two hours the ratio of rubber to glue was fairly high, approximately 7 to 1. Frequent additions of water and occasional additions of ammonia were employed during the process. The ammonia seemed to serve the purpose of a peptizing agent, the glue that of a protective colloid for rubber.

Numerous equivalents of glue were found which could be used as protective colloids, including sodium resinate, gum arabic, gluten, sodium alginate, blood albumen and casein. In fact it would appear that the requirements of the protective colloid layer are high water absorption and a fair degree of jelly strength and of viscosity.

In case the batch is permitted to lose water by evaporation during milling, or in case the rubber strips are added too rapidly, a sudden in-

⁴ U. S. P. 1,498,387, June 17, 1924.

version of phase occurs, to give a true rubber batch, that is, one in which the rubber is the continuous and the aqueous paste the discontinuous, phase. Further working of this batch with aqueous paste results again in dispersion. Thus the process of coagulation of rubber dispersions is shown to be a reversible rather than an irreversible process,⁵ a viewpoint easily comprehended by colloid chemists. The power chart shown in Figure 1 illustrates in terms of the energy of mastication the difference between rubber in the continuous phase and in the discontinuous phase.

The work of the last five or six years, including that of W. B. Pratt⁶

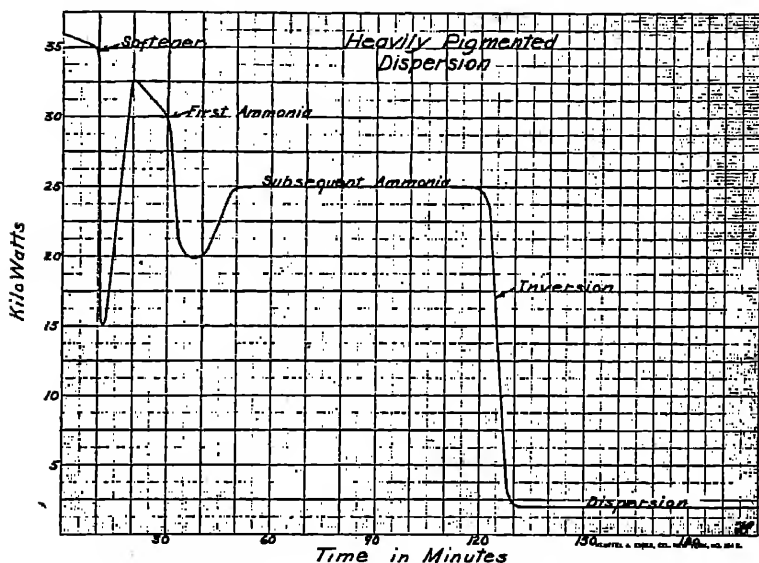


Fig. 1.—Dispersion of a Rubber Compound—Power Chart.

and his associates, has served still further to simplify the technic of producing rubber dispersions. At the present time, largely as a result of the work in Pratt's laboratory, the rubber industry has adopted as standard for the production of rubber dispersions a particular type of internal mixer, namely, the Baker-Perkins mixer equipped with blades especially designed for the purpose (Figs. 2 and 3). By means of this mixer the rubber compounder is permitted to prepare rubber dispersions from a very wide range of rubber "compounds." This range even in-

⁵ The view that latex could never be made artificially has been attributed to Mr. Kaye by Tuttle, *India Rubber World*, 68, 213 (1928).

⁶ W. B. Pratt, *Ital. P.* 225,949, June 12, 1923; *Eng. P.* 233,370 (1923); U.S.P. 1,671,314, May 29, 1928. J. G. Richards and Geo. P. F. Smith, U.S.P. 1,671,316, May 29, 1928.

cludes compounds in which pigments predominate and rubber plays a relatively minor part. It is also permitted the compounder to substitute the major part of rubber by reclaim in a given recipe, without in any way preventing the process of dispersion. In fact, "reclaim dispersions," as these batches are called, are prepared in a shorter time and with less uncertainty than rubber dispersions.

Dispersions containing as high as seventy-five per cent by weight of total solids have been prepared. These can be diluted with water to any

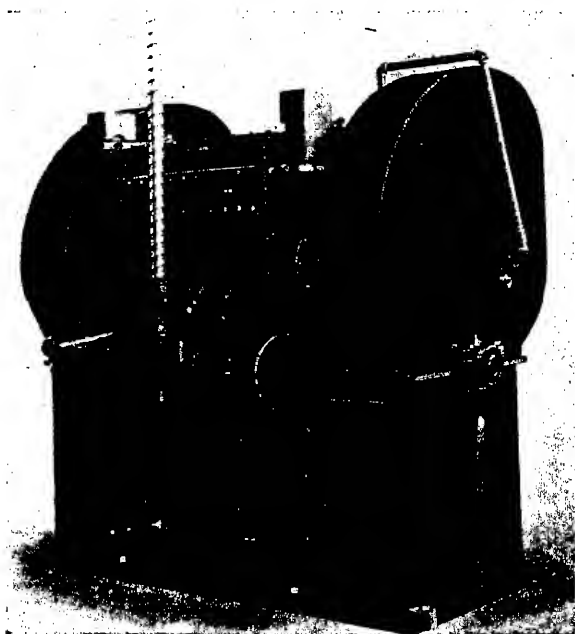


FIG. 2.—Baker-Perkins Mixer.
50 gal. capacity.

desired consistency, and in either concentrated or dilute form remain stable for long intervals just as latex does.

From the power chart (Fig. 1) it is obvious that the initial operation which occurs in the internal mixer is a gradual softening due to the masticating action of the mill. Only after the thorough incorporation of the protective colloids and water can the batch be inverted to form a dispersion. If the initial working of the rubber is insufficient, if the admixture of the colloids is hurried and finally, if the water is added in too large increments, lumpy dispersions will result.

Although it is not the purpose of the author in this paper to discuss the uses of rubber dispersions it is of interest to mention that a number of applications have already been introduced into the rubber industry. For the most part these are associated with the spreading of fabric and have resulted in advantages over rubber cement in reduction of fire hazard, improved adhesions between the treated fabric and other rubber constructions, possible economy of compounding through the use of

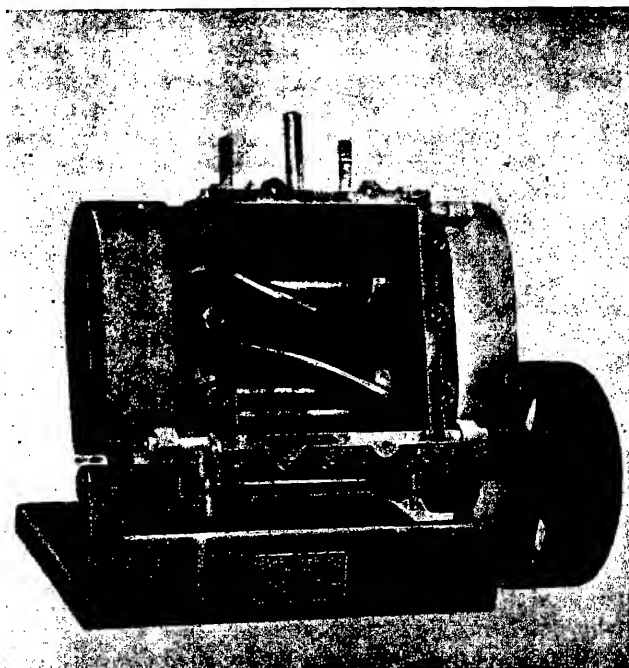


FIG. 3.—Baker-Perkins Mixer.
Laboratory Size.

reclaim, greater speed in spreading due to the elimination of fire hazards, economy in labor costs, and ultimate improvement in the quality of finished goods through one of the above advantages. It is pertinent that the new product presents certain unique advantages and that the possibilities of extending the uses of aqueous dispersions in the rubber industry are more than hopeful. With the object of promoting these uses a new company⁷ has recently been incorporated.

⁷ Dispersions Process, Inc., Oaks, Penna.

PROPERTIES OF RUBBER DISPERSIONS

The points of resemblance and of difference between rubber dispersions and latex can best be described by first mentioning the differences between masticated and crude rubber at room temperature.

Masticated rubber is soft, tacky, highly plastic, transparent, and appears practically structureless. It can readily be softened by hydrocarbon solvents and easily goes into solution to give a cement dispersion with a concentration of twenty per cent by weight.

Crude rubber, first latex, for example, is hard, non-tacky, tough and opaque, and superficially appears to have a nodular structure. Cement dispersions of first latex in rubber solvents at concentrations as low as five per cent have a high viscosity and require several hours on a laboratory shaker to give smooth consistency. It is believed that the resistance to solvent swelling and dispersion is to be attributed to the shell structure of the former latex particles which in the case of unmasticated rubber have not been disturbed. In masticated rubber, however, not only have these shells been distorted, but the particles themselves have been to some extent disrupted. Thus the solution effect of swelling solvents proceeds much more smoothly and quickly for masticated than for unmasticated rubbers.

In terms of the three-shelled structure for latex particles advocated by Hauser and Freundlich,⁸ mastication has split the shells of protein and of hard hydrocarbon and has permitted the soft hydrocarbon to be extruded under pressure. That this shell-cracking involves only a portion of the latex globules even in highly masticated rubber would seem to be indicated from unpublished microscopic studies. It stands nevertheless that mastication has produced a profound change in the rubber mass and this change must to some extent involve the properties of even those globules which have not been ruptured.

Crude rubber dispersed in water solutions might be expected to show similarities to latex but also some differences from it and that is just what is found. That the mastication of the rubber modifies its properties and that this modification of properties persists even in the dispersed particles themselves is evidenced from the following:

1. Dispersions do not show particles of characteristic shapes such as the pear-shaped particles of latex.
2. Although the size of the particles is roughly the same in both cases, the quality of the dried film is different, in texture, in tackiness and in strength.
3. The quality of the dried film is subject to the control of the chemist making the dispersion.

⁸ *Kolloid-Z.*, 36, 15 (1925).



FIG. 4.—Latex. $\times 1000$.



FIG. 5.—Dispersed Plantation Rubber. $\times 1000$.

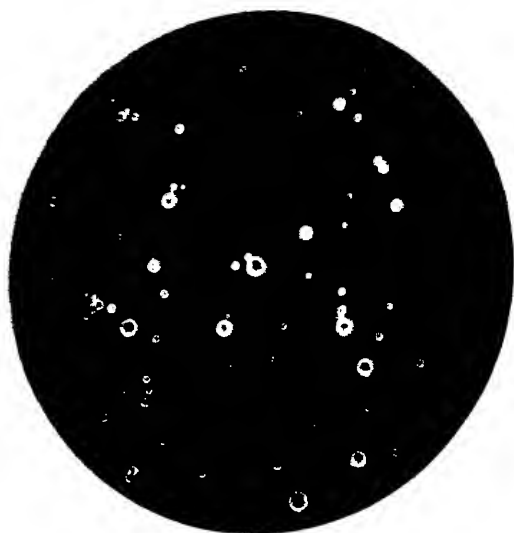


FIG. 6.—Water Dispersed Benzene Cement. $\times 1000$.



FIG. 7.—Same cement as in Fig. 6, showing some of larger particles. $\times 1000$.

In order to illustrate the characteristic differences between the various types of aqueous dispersions of rubber, it will be noted first, in Figure 4, that latex displays a characteristic particle shape, with not only a departure from the spherical, but with some tendency for the larger particles to display appendages. In an aqueous dispersion of crude rubber (Fig. 5) the particles show about the same average size as in latex, but with less characteristic shape, and less departure from sphericity. In Figure 6 the particles of a benzene rubber cement dispersed in water appear perfectly spherical. The tendency of these dis-



FIG. 8.—Reclaim Dispersion. $\times 1000$.

persions to cream is probably explained by the presence of larger particles such as those shown in Figure 7.

That aqueous dispersions of an auto tire reclaim are composite in character is apparent from the variety of particles shown in Figure 8. It is noteworthy that the average particle size is fully as small as that of latex. As might be expected from our knowledge of latex, the smaller particles in all the dispersions above mentioned show active Brownian movement.

In a new field there are obviously many problems which confront the theoretical as well as the practical chemist and which call for considerable experimentation. One of the questions which has not been answered entirely satisfactorily is why the particles of dispersed rubber

should have approximately the same size as those of latex, and this independently of whether the dispersion is made on a smooth-roll mixer or in an internal mixer. We know only a part of the answer. One might be tempted to reason in terms of the Hauser structure for latex particles so far as crude unvulcanized rubber is concerned, even though we have good evidence that the particle is vastly modified by mastication. But just why should approximately the same-sized particle be produced in the case of mixes consisting almost entirely of reclaim? Another question is how some protective colloids, even though present in very small amounts, leave films of rubber which are comparatively non-tacky, whereas others will permit the formation of films with satisfactory building tack.

SUMMARY

By a mastication method there have been prepared dispersions of rubber and of reclaim. Rubber dispersions have been shown to possess properties different from latex and from dispersions of rubber cements. The method of masticating a plastic substance like rubber in the presence of a hydrophilic colloid and water constitutes a new method of procedure in the preparation of aqueous dispersions.

*Research Chemical Laboratory,
B. F. Goodrich Co.,
Akron, Ohio.*

STUDIES OF ORGANOPHILIC COLLOIDS

BY G. S. WHITBY, J. G. McNALLY AND W. GALLAY

The viscosity of sols of organophilic colloids has been attributed in part or wholly to one or more of the following factors, namely: (a) solvation, (b) structure, (c) a charge on the micelles. Kruyt in particular¹ has emphasized the last mentioned factor as contributing to the viscosity of sols, not merely of hydrophilic, but also of organophilic colloids. Kruyt and Eggink² observed an electro-viscous effect even in the case of a benzene sol of rubber, *i.e.*, in the case of a hydrocarbon colloid dispersed in a hydrocarbon medium, the viscosity being lowered by the addition of acids and of mercuric chloride and raised by the addition of ammonia. Whitby and Jane³ found that the viscosity of such a sol was lowered markedly, not only by minute amounts of organic acids, but also by minute amounts of strong organic bases; and, further, that the effectiveness of both acids and bases was in the order of their dissociation constants as determined in water. Humphrey and Jane⁴ showed by a cataphoresis experiment that in a benzene sol of rubber prepared without very special precautions to exclude moisture both positively- and negatively-charged particles exist, but that when great care is taken to exclude moisture charged particles are entirely absent. They conclude that the previous observations on the effect of acids and bases on the viscosity of rubber sols do not imply a true electro-viscous effect. More recently it has been found by the present authors that no reduction whatever in viscosity is produced by the addition of acids or bases to sols of meta-styrene in benzene—a hydrocarbon system very similar in general to the rubber-benzene system. Hence it seems fair to conclude that, although in some cases a charge on the micelles may contribute a certain amount to the viscosity of sols of organophilic colloids, it is not a universally present or essential factor in the viscosity and stability of such sols.

¹ "Colloids," New York, Wiley & Sons, Inc., 1927.

² *Proc. Roy. Acad., Rotterdam*, 26, 43 (1928); Eggink, *Rec. trav. chim.*, 42, 317 (1923).

³ "Colloid Symposium Monograph," Vol. 2, New York, The Chemical Catalog Co., Inc., 1925, p. 16.

⁴ *Kolloid-Z.*, 41, 293 (1927).

It remains therefore to consider the other two factors which have been regarded as accounting for the high viscosity of sols of lyophilic colloids, *viz.*, solvation and structure. Some authors, *e.g.*, Hatschek,⁵ consider that the viscosity is wholly accounted for by solvation; others, *e.g.*, McBain,⁶ consider that solvation is utterly inadequate to account for the high viscosity and regard the latter as chiefly due to some kind of structure arising from the presence of ramifying aggregates of micelles. The present authors have followed several lines of experiment, with the object, *inter alia*, of throwing light on the question as to whether solvation or structure is chiefly responsible for the high viscosity of sols of organophilic colloids; and some consideration will now be given to these, although no entirely conclusive answer can yet be given to the question.

PRECIPITATION EXPERIMENTS

In experiments on the precipitation of benzene sols of meta-styrene by ethyl alcohol and in experiments on the precipitation of benzyl alcohol sols of cellulose acetate by tertiary butyl alcohol, it was found that, for sols of any given specimen, the higher the concentration of the sol, the smaller was the proportion of precipitant required. Mardles⁷ found similarly that the volume of light petroleum required to precipitate sols of cellulose nitrate and of cellulose nitracetate in acetone was smaller, the greater the concentration of the sol. Further, the authors found on comparing samples of meta-styrene representing different degrees of polymerization, that samples yielding more viscous sols at a given concentration required less precipitant than samples yielding less viscous sols. Hence it seems reasonable to conclude that, the smaller the proportion of bound solvent in a sol of a given organophilic colloid, the smaller is the proportion of the given precipitant required to produce separation.

When precipitation experiments were carried out with sols of cellulose acetate at different temperatures, it was found that for a given sol the volume of precipitant required to produce separation increased rapidly with rise in temperature. Thus, for example, a 1 per cent sol of cellulose acetate in benzyl alcohol required for precipitation at 40° C. 45 cc. and at 60° C. 132 cc. of tertiary butyl alcohol. Hence it might be considered in the light of the conclusion formulated at the close of

⁵ *J. Phys. Chem.*, 31, 383 (1927); J. Alexander, "Colloid Chemistry," Vol. 1, New York, The Chemical Catalog Co., Inc., 1926, p. 738.

⁶ *J. Phys. Chem.*, 30, 239 (1926).

⁷ *J. Soc. Chem. Ind.*, 42, 123T (1923).

the preceding paragraph that the amount of solvent bound in the disperse phase was greatly reduced by the rise of temperature. Yet, as pointed out later, the relative viscosity of the sol suffers far less change with change of temperature than does the readiness with which precipitation can be brought about. Thus, for example, the cellulose acetate sol just quoted changed in relative viscosity between 40° C. and 64° C. only from 2.11 to 1.97. It is probable that precipitation by a non-swelling agent affords a method of comparing sols in regard to the proportion of unbound solvent or conversely in regard to the amount of solvation only under certain restricted conditions, as for example in the comparison of sols of different concentrations at a given temperature in a given dispersion medium, or in the comparison of different specimens of a given organophilic colloid in a given medium. It is probably not generally applicable in comparing sols of a given colloid in different media (see later) or in comparing sols of a given colloid in the same medium at different temperatures. For the precipitation is determined by the force of attraction of the precipitant on the one hand and the colloid on the other for the solvent. And the force of attraction of a given precipitant for different solvents and of different precipitants for a given solvent will vary, as recent work by Magden, Peel and Briscoe^a on the effect of mixing organic liquids, clearly indicates. It is also probable that the relative force of attraction of a given precipitating liquid for a given solvent will not be the same at different temperatures.

In connection with the use of precipitation experiments in the study of sols of organophilic colloids, the following further remarks may be added. Mardles^b found a rough parallelism in certain cases between the viscosity of cellulose acetate sols in binary mixtures of different composition and the ease of precipitation, mixtures yielding sols of minimum viscosity corresponding approximately with those yielding sols requiring the greatest amount of a given precipitant. In some cases, however, *e.g.*, sols of cellulose acetate in mixtures of benzyl alcohol and acetone, there was no parallelism whatever between the viscosity and the ease of precipitation. In experiments on the precipitation of sols of meta-styrene of the same concentration in eighteen single liquids the present authors found that there was no general parallelism between viscosity and ease of precipitation, and, further, that the order of the ease of precipitation for any one coagulant, *e.g.*, ethyl alcohol, was not the same as that for another, *e.g.*, octane. Particularly striking excep-

^a *J. Chem. Soc.*, 1928.

^b *J. Soc. Chem. Ind.*, 42, 207T (1923).

tions to any parallelism between the viscosity and the ease of precipitation were sols of meta-styrene in methylal and acetal. These sols had the lowest viscosities in the series of eighteen sols, yet they were the most readily precipitated by each of the five precipitants used.

VISCOSITY-TEMPERATURE RELATIONS

Systematic observations on the change with temperature of the viscosity (relative to the viscosity of the pure solvent at the same temperature) of sols of lyophilic colloids are on record in only a few cases. For a 9.39 per cent sol of sodium caseinate, having at 25° a viscosity 23.72 times the viscosity of water, Chick and Martin¹⁰ found the relative viscosity to fall 92.8 per cent when the temperature was raised from 0° C. to 99.6° C. (f^{11} at 0° C. and 99.6° C., 91 per cent and 48.5 per cent respectively). For a 1.6 per cent sol of the same material the fall in viscosity between 17° C. and 50° C. was only 1.18 times greater than for water. Davis and Oakes¹² found the fall in viscosity of a 1 per cent gelatin sol with rise of temperature to be very small. Loeb found that the fall in viscosity of gelatin sols with rise of temperature was relatively greater at higher than at lower concentrations, as the following approximate figures taken from one of his graphs indicate.¹³ Similarly data given by Mardles¹⁴ show incidentally that

TABLE I. *Change of Relative Viscosity of Gelatin Sols with Rise of Temperature.*

Concentration (per cent).....	1	2	5
Fall (per cent) in relative viscosity over range 25° C. to 60° C.....	16.5	26	53

the change of viscosity with temperature of sols of cellulose acetate in triacetin is relatively greater at higher than at lower concentrations, as the following figures calculated from his data demonstrate.

TABLE II. *Change of Relation Viscosity of Cellulose Acetate Sols in Triacetin with Temperature.*

Concentration (per cent).....	1	2	5
Change in Relative {10° to 30°.....	24	..	46
Viscosity (per cent) {10° to 30°.....	3.3	19	25

¹⁰ *Kolloid-Z.*, 11, 102 (1912).

¹¹ See later.

¹² *J. Am. Chem. Soc.*, 44, 464 (1922).

¹³ Loeb, "Proteins and the Theory of Colloidal Behavior," New York, McGraw-Hill Book Co., 1948, p. 266.

¹⁴ *J. Chem. Soc.*, 1923, 1951.

The present authors found a similar relationship between the temperature coefficient of viscosity and the concentration with sols of cellulose acetate in other liquids, as the following data will indicate.

TABLE III. *Change of Relative Viscosity of Sols of Cellulose Acetate with Temperature.*

Solvent	Concentration (Grams per 100 cc. Solvent)	Tem- perature Range °C.	Relative Viscosity at Lower Tem- perature	Change in Relative Viscosity Per Cent	<i>f</i> Per Cent
Phenyl ethyl alcohol....	1	45-64°	2.11	6.6	14.6-11.9
Phenyl ethyl alcohol....	2	45-64°	3.48	10.6	36.1-31.6
Benzyl alcohol	1	30.5-64°	2.77	16.6	24.2-18.25
Benzyl alcohol	2	30.5-64°	6.82	32.4	57.8-47.8
Acetone	1	9-33.7°	3.85	22.1	40.6-29.6
Acetone	2	9-33.7°	14.90	34.5	81.3-72.2
Cyclohexanone	1	9-33.7°	4.42	29.6	46.2-31.6
Cyclohexanone	2	9-33.7°	13.55	40.1	79.8-67.5

A sol of polyvinyl acetate in benzene showed the following temperature-viscosity relations. The fall in relative viscosity over the range

TABLE IV. *Polyvinyl Acetate in Benzene.*

(2 grams per 100 cc. benzene.)

Temperature (°C.)	15	25	35	45	55
Relative viscosity	4.63	4.51	4.50	4.32	4.28
<i>f</i> (per cent).....	47.8	44.8

15° C. to 55° C. is only 7.7 per cent. A sol of rubber (0.328 gram per 100 cc. benzene), having a relative viscosity of 3.58 at 25° C., increased in relative viscosity only 7.5 per cent over the range 11° C. to 45° C. (*f* at 11° C., 35.4 per cent). A sol of meta-styrene (0.328 grams per 100 cc. benzene), having a relative viscosity of 3.52 at 25° C. suffered no change whatever in relative viscosity over the range 15° C. to 55° C. (*f*, 37.5 per cent).

In connection with such results several points offer themselves for comment. The change of relative viscosity with temperature is surprisingly small in the case of several of the sols quoted above. In the face of this it is difficult to credit the view that the main factor in the viscosity of such sols is the existence of a structure. Any structure present in a sol of an organophilic colloid would presumably be similar in character to that in a jelly, since, as Mardles¹⁵ has pointed out, there

¹⁵ *Trans. Faraday Soc.*, 18, 365 (1922).

is no abrupt transition from sol to jelly. Yet jelly structure is sensitive to rise in temperature, and any corresponding structure in a sol might reasonably be expected to be more fragile and more sensitive to temperature than the structure in a solid jelly. Further, there is no evidence that the viscosity of gelating sols is more sensitive to temperature than that of non-gelating sols. It was found that the temperature coefficient of the viscosity of sols of cellulose acetate in phenyl ethyl alcohol and in benzyl alcohol, which gelate at room temperature, is not greater but rather less than that of sols of the same material in acetone and in cyclohexanone which do not gelate. This is true even when the temperature of the former is lowered to very near the gelation temperature.

Although, on comparing sols of a given colloid in a given liquid, the sensitivity of the relative viscosity to temperature change is greater, the higher the concentration, and hence the greater the proportion of bound solvent, there would not appear to be, as between sols of different colloids or sols of the same colloid in different liquids, any universally applicable relation between the proportion of bound solvent and the magnitude of the temperature coefficient. In other words a reduction in the degree of solvation with rise of temperature takes place with different degrees of readiness in different cases. The figures for f given above represent the percentage volume occupied by the disperse phase as calculated from Hatschek's equation, $f = (\eta - \eta_0/\eta)^3$. The viscosity of sols of meta-styrene, rubber and polyvinyl acetate in benzene is, it will be observed, less sensitive to temperature change than the viscosity of sols of cellulose acetate in which the disperse phase occupies (on the basis of Hatschek's equation) a similar volume. This is true despite the fact that, since the concentration is lower, the degree of solvation (f/c) is greater in the former than in the latter sols.

It appears probable that when, as in the case of Chick and Martin's sodium caseinate sol, and in the cases, above quoted, of 2 per cent sols of cellulose acetate in acetone and in cyclohexanone (Table III) the volume of the disperse phase is in excess of that corresponding to close packing of the micelles, the viscosity is particularly sensitive to temperature changes.

SWELLING AND VISCOSITY

The relative viscosity of sols of rubber of the same concentration in twenty-eight different liquids (hydrocarbons, esters, ethers, amines and alkyl halides) was determined, and the swelling of rubber, both raw and vulcanized, in the same liquids was measured. No general

parallelism between swelling and viscosity could be discovered. If, however, only liquids of a similar chemical character, such as members of a homologous series, were considered, a parallelism was noticeable. For example, in the series benzyl chloride, benzal chloride and benzo-trichloride, and again in the series benzene, toluene, xylene, both swelling and viscosity increased regularly.

In no case does the amount of a liquid which it is possible to determine by direct measurement as being imbibed by massive pieces of colloids such as rubber and meta-styrene approach the amount which Hatschek's equation for the viscosity of emulsoids, $\eta = \eta_0 / 1 - \sqrt[3]{f}$, would indicate as being bound in the disperse phase. Thus, for example, over the range of concentrations where, using Hatschek's equation, the volume of solvent bound by 1 gram of the solute is constant, this volume is in the case of benzene sols of rubber and of meta-styrene about 160 and 135 respectively. Yet the volume found to be imbibed by these colloids by immersing pieces of them in benzene and noting the increase in weight is of the order of 30 and 6 volumes only, respectively. It is, however, doubtful whether direct measurements of swelling afford any reliable information as to the volume of solvent which a colloid is capable of binding when dispersed in a sol. When a piece of raw, unmilled rubber is placed in benzene or other solvent and left undisturbed, it imbibes the liquid and swells, in most cases quickly at first and then more gradually; but before imbibition has ceased and equilibrium has been attained, diffusion of the hydrocarbon from the swollen mass begins and the weight begins to fall. In the case of meta-styrene dispersion of the colloid from the surface of the swollen mass begins after swelling has proceeded to a much smaller extent than is necessary for the dispersion of rubber to begin. Probably, in the case of rubber, dispersion would similarly begin after a relatively small amount of swelling were it not for the protein network in which the hydrocarbon particles are enmeshed. It seems, then, that the amount of solvent bound by such colloids when dispersed in a swelling agent may be much greater than the maximum amount which it is possible to determine as being imbibed by a massive piece, and may conceivably be of the order which Hatschek's equation would indicate. In this connection the following experiment may be noted. Acetone was slowly added with stirring to a 1 per cent benzene sol of rubber until a permanent, faintly visible precipitate appeared. The precipitate, which was allowed to settle and was separated, was found to contain approximately 10 volumes of benzene to 1 volume of rubber. This would appear to represent the minimum amount of solvation necessary to maintain rubber in dispersion. The

actual degree of solvation in the original sol is very much greater than this, since prior to the appearance of the precipitate the sol had, as a result of the addition of the de-solvating agent, fallen in viscosity so greatly that it differed only slightly in viscosity from the pure liquid and since it showed a Tyndall effect.

McBain's view that structure is chiefly responsible for the high viscosity of sols of organophilic colloids is based largely on sorption experiments, in which he finds, for example, that cellulose nitrate takes up only its own weight of solvent. It must, however, be considered as doubtful whether sorption experiments can give an adequate idea of the magnitude of solvation in a sol, since in the latter all the solvent influenced by the micelles must be regarded as bound. In this connection it may be pointed out that sorption by vulcanized rubber when placed in the vapors of organic liquids is independent of the degree of vulcanization,¹⁶ yet, as is well known, imbibition by vulcanized rubber immersed in organic liquids is greatly influenced by the degree of vulcanization; and, more important, sorption by raw rubber from the vapors of organic liquids is not affected by mastication¹⁷—a treatment which affects vastly the viscosity of sols prepared from the material.

COLLOIDAL PROPERTIES AND DEGREE OF POLYMERIZATION

Studies of artificial organophilic colloids prepared by the polymerization of simple molecules show that in the case of any one colloid the colloidal properties are dependent on the molecular magnitude of the specimen. It is found that the viscosity of sols of a given concentration is greater the higher the degree of polymerization, in illustration of

TABLE V. *Samples of Polyvinyl Acetate.*

Molecular Weight of Sample *	Degree of Polymerization (Approximate number of monomeric molecules)	Relative Viscosity at 25°C. (0.328 grams per 100 cc. benzene)
400.....	5	1.303
462.....	5	1.333
747.....	9	1.561
1068.....	12	2.304
1150.....	14	2.725
1450.....	17	3.700
3200.....	37	5.878
5000.....	60	8.389

* In bromoform. The results in benzene were similar.

¹⁶ Kirchhof, *Kolloidchem. Beihefte*, 6, 1 (1914).

¹⁷ Pohle, *Kolloidchem. Beihefte*, 13, 1 (1920).

which the data for eight specimens of polyvinyl acetate obtained by fractionation may be quoted. (See Table V on opposite page.) In the case of meta-styrene, a colloid which shows elastic properties at elevated temperatures (see later), such properties were found to be markedly influenced by the degree of polymerization. Meta-styrene obtained by the auto-polymerization of styrene at room temperature and representing a very high degree of polymerization (observable depression of freezing point: nil), shows very much better elastic properties, *e.g.*, in regard to the completeness of recovery from deformation, than meta-styrene prepared by heating styrene and representing a relatively low state of polymerization (molecular weight: say, 2000).

ELASTIC PROPERTIES

Several organic colloids which are hard and even brittle at room temperature exhibit, it has been found, elastic properties at slightly elevated temperatures. One of the writers has previously referred to several such colloids.¹⁸ Another example, encountered more recently, is polymerized vinyl acetate. The following experiments will illustrate the elastic behavior of this material, the sample used having a molecular weight of 1555. (a) At 50° C. a strip 3.2 cm. long was extended to 90 cm. (2710 per cent elongation) without breaking, and when released contracted under no load to 13 cm. (350 per cent elongation) in 10 minutes. (b) A strip 7 mm. wide, 0.8 mm. thick and 3.2 cm. long was subjected to a load of 75 grams (1.34 kilograms per sq. cm.), and the temperature was raised gradually over a period of 30 minutes from 15° C. initially to 41.5° C. finally. The following Table shows the elongation at various temperatures.

TABLE VI. *Effect of Temperature on Elongation.*

Temperature (°C.)	15	19	22	27	31	34	35	36	37	
Elongation (per cent)...	0	3	12.5	25	40	56	72	87	103	
Temperature (°C.)	37.5	38	38.3	38.8	39.2	40.0	40.2	40.6	41.0	41.5
Elongation (per cent)	119	134	150	181	212	275	368	466	525	710

On releasing the strip at a temperature of 41.5° C., it contracted to 6.5 cm. (103 per cent elongation) in 10 minutes. It will be observed that the temperature at which the material becomes readily extensible is approximately 40° C.

¹⁸ Whitby, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 204.

Auto-polymerized styrene shows better elastic properties than the sample of polyvinyl acetate just mentioned, recovery from extension being more rapid and complete. An extensive study of this material, to be published elsewhere in detail, shows clearly that above its "elasticity temperature," which is in the neighborhood of $65^{\circ}\text{C}.$, it behaves essentially as does raw rubber, which has a lower "elasticity temperature," at ordinary temperatures. It shows set, elastic after-effect, creep, and the phenomena associated with "racking." When it is kept in an extended condition above its "elasticity temperature," the deformation gradually changes, as in raw rubber, from being elastic in character to being irreversible, and this change in the character of the deformation occurs more quickly the higher the temperature.

A sample of auto-polymerized styrene, extended 1300 per cent, and one of polyvinyl acetate, extended 1500 per cent, which subjected to an X-ray examination, showed no diffraction pattern. Hence it would appear that the orientation which is revealed as being present in rubber at much more moderate extensions by the existence of a pattern on the X-ray diagram is not essential to the possession of elastic properties by organic colloids.

The artificial elastic colloids, polyvinyl acetate and meta-styrene, are highly heterogeneous, being composed of mixtures of an unbroken series of polymers of different molecular magnitudes. Thus, a sample of polyvinyl acetate was separated into four fractions ranging in molecular weight from 566 to 6192. Now it has been supposed recently by several writers that the hydrocarbon of rubber consists of two sharply distinct phases, which have been designated "sol" and "gel" rubber, and correspond to the "soluble" and "pectous" forms of rubber of which Caspari wrote a considerable time ago.¹⁹ The present authors, however, believe²⁰ that natural caoutchouc is heterogeneous in a way similar to that in which polyvinyl acetate and meta-styrene are heterogeneous, and in which synthetic rubber²¹ has been found to be. The so-called "sol" phase of rubber has been looked upon as that portion of the rubber which will diffuse out of the swollen mass when rubber is allowed to stand in ether, the "gel" phase being that portion which is left behind. Experiments by the authors show, however, that there are not two sharply distinct phases in rubber, since the proportion of "gel" rubber determined in a given sample of raw rubber depends on the swelling agent used, and, further, since the "gel" phase is itself heterogeneous. If benzene is used, a higher proportion of "gel" rubber

¹⁹ *J. Soc. Chem. Ind.*, 32, 1041 (1913).

²⁰ Cf. Whitby, *loc. cit.*, p. 221.

²¹ Whitby and Crozier, unpublished.

is obtained than if ether is used; if petrolic ether is used, a lower proportion is usually obtained, while if a little piperidine, diethylamine or other strong organic base (which has the effect of increasing the swelling^{21a}) is added to petrolic ether an increased proportion is secured. As showing the heterogeneous character of the "gel phase," it was found that as diffusion proceeds successive fractions yield sols which at the same concentration possess different viscosities.

As the above remarks will suggest, it seems probable that study of artificial elastic colloids, which can be prepared in various states of polymerization, will assist considerably in understanding the cause of the elasticity of the important natural elastic colloid, rubber.

CHEMICAL CONSTITUTION AND SWELLING

In an earlier paper²² it was stated that in general colloids, such as cellulose acetate, the molecules of which are of a polar character imbibe most readily polar organic liquids, and conversely that hydrocarbon and other non-polar colloids, such as rubber, swell most in liquids of a non-polar character. Numerous examples were quoted in support of this generalization. The behavior of polyvinyl acetate may be instanced as a further example which has since been studied. It has been found that, in agreement with the above generalization, this colloidal ester swells and disperses generally in polar organic liquids. It dissolves readily in the lower members of the series of fatty acids, ketones, alcohols and esters, and less readily in the higher than in the lower members of such series. It also dissolves in such liquids of notably high dielectric constant as acetonitrile and nitromethane. A few facts may be mentioned in illustration. Polyvinyl acetate disperses rapidly in methyl and ethyl formates; rapidly, but not so rapidly, in methyl and ethyl acetates. It disperses less rapidly in ethyl oxalate; still less rapidly in butyl oxalate. It is soluble in methyl and ethyl alcohols; swells without dispersing in butyl and amyl alcohols at room temperature; does not swell in hexyl and caprylic alcohols. It swells but does not dissolve in ethyl ether, and does not swell in butyl ether. Methylal produces rapid dispersion; acetal only slight swelling.

Several examples which accord with the generalization laid down are to be found in the work which Staudinger and his pupils have lately conducted on polymeric products. The colloid obtained from the highly polar molecule, acrylic acid, $C_2H_3.CO_2H$, is soluble in water and

^{21a} Whitby and Jane, *loc. cit.*

²² Whitby, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 204.

formamide, and insoluble in acetone and alcohol. If it is converted into the less polar ester, $(C_2H_5 \cdot COOC_2H_5)_x$, the product is found to be soluble in benzene and acetone, and to be precipitated by water, ether and alcohol. When the ester is converted into the tertiary alcohol, $(C_2H_5 \cdot C(OH)Me_2)_x$, which, owing to the presence of a hydroxyl group, is more polar, a product is obtained which is soluble in alcohol and insoluble in benzene. If now the alcohol is reduced to the non-polar hydrocarbon, $(C_2H_5 \cdot CHMe_2)_x$, the product is soluble in benzene and precipitated by alcohol.²³

*McGill University,
Montreal, Quebec, Canada.*

²³ Urech, Zürich thesis, 1927.

THE INFLUENCE OF ELECTROLYTES AND NON-ELECTROLYTES UPON THE OPTICAL ACTIVITY AND RELATIVE RESISTANCE TO SHEAR OF GELATIN SYSTEMS

BY J. R. FANSELOW

In spite of the large amount of work that has been carried out in the study of the physical properties of gelatin systems there still remains the controversy as to whether the properties of these systems can best be explained by the classical laws of stoichiometry or by the laws obtained from the behavior of heterogeneous (colloidal) systems. Studies of the changes in degree of dispersion in gelatin systems with variation of temperature and electrolyte concentration and studies of the mechanism of the sol-gel transformation have not been particularly conclusive.

A general criticism that may be applied to a large amount of the physico-chemical investigations of gelatin is that one or more of the important factors influencing the property under consideration has been neglected or that the studies have not been sufficiently extensive to give an adequate perspective. Loeb made a valuable contribution by emphasizing the necessity of controlling the hydrogen-ion activity in the investigations of the influence of different electrolytes upon the properties of gelatin systems, but he missed two most important observations by failing to extend his studies to wider ranges of hydrogen-ion activity and by failing to permit his systems to reach equilibrium at low temperatures before making his viscosity measurements. Many other investigators have failed in their studies to consider the fact that the influence of electrolytes upon the protein systems is markedly altered by different hydrogen-ion activities.

The present investigation was conducted for the purpose (1) of determining whether gelatin systems in which the hydrogen-ion activity is controlled do not exhibit the effect of specific ion influence or that the evidence is only complicated by the lyophilic character and the amino acid nature of the protein substance, and (2) of obtaining more information concerning the influence of some factors which alter the sol-gel transformation of gelatin systems. Optical activity and relative resistance to shear were selected as properties for study because it is generally believed that optical activity reflects deep seated changes in the

intramolecular structure of substances, while relative resistance to shear serves as a means of evaluating the accompanying macrophysical changes of the systems. Space in this paper will not permit the presentation of the details of the study so only a brief of the results obtained will be presented at this time.

INFLUENCE OF TEMPERATURE AND GELATIN CONCENTRATION UPON SYSTEMS AT DIFFERENT HYDROGEN-ION ACTIVITIES

When the specific rotation of gelatin systems at different temperatures with acetic acid or sodium hydroxide is plotted against the pH values two types of curves are obtained.¹ Those at temperatures of 30° C. or higher show a minimum at the isoelectric point and a discontinuity in the region of pH 8. This type of curve is characteristic of systems at temperatures above 30° C. The specific rotation decreases slightly with increase in temperature, and is, within experimental error, independent of the gelatin concentration. A calculation of the apparent relative volume of the dispersed phase by means of Kunitz modification of Einstein's equation,²

$$\eta = \frac{1 + 0.5\varphi}{(1 - \varphi)^2},$$

where η = relative resistance to shear,

φ = volume of dispersed phase in unit volume of the system, and

indicates that the variation of degree of hydration with change in hydrogen-ion activity is closely parallel with the variation of specific rotation, and that the value of φ/C (C = concentration of gelatin in grams per 100 cc.) does not vary appreciably with temperature or gelatin concentration at temperatures above 30° C. This leads to the view that the gelatin micellae at these temperatures are highly dispersed, independent individuals unattached to one another.

At temperatures below 25° C. a very different type of specific rotation curve is obtained. This type of curve is characteristic of series of systems at temperatures between 25° C. and 3° C. (or lower) and is paralleled, in this and all other series of systems with variation of electrolyte concentration, by a similar type of curve for relative resistance to shear.³ At these temperatures, instead of the gradual increase in specific rotation and relative resistance to shear on either side of the isoelectric point, as at 30° C., there is a very abrupt increase in relative resistance to shear and an increase, though not so marked, in specific rotation. Farther from the isoelectric point, however, the magnitude of

¹ Kraemer and Fanslow, *J. Phys. Chem.*, 32, 894 (1928).

² M. Kunitz, *J. Gen. Physiol.*, 9, 715 (1925-26).

³ Kraemer, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 102; Kraemer and Fanslow, *J. Phys. Chem.*, 32, 894 (1928).

both properties decreases, but in the region of pH 8, corresponding to the minimum in these properties at 30° C., there is a slight increase in both properties. The Tyndall intensity curve for these systems⁴ does not vary from that at 30° C. except to increase appreciably in the immediate region of the isoelectric point. The specific rotation of the systems at

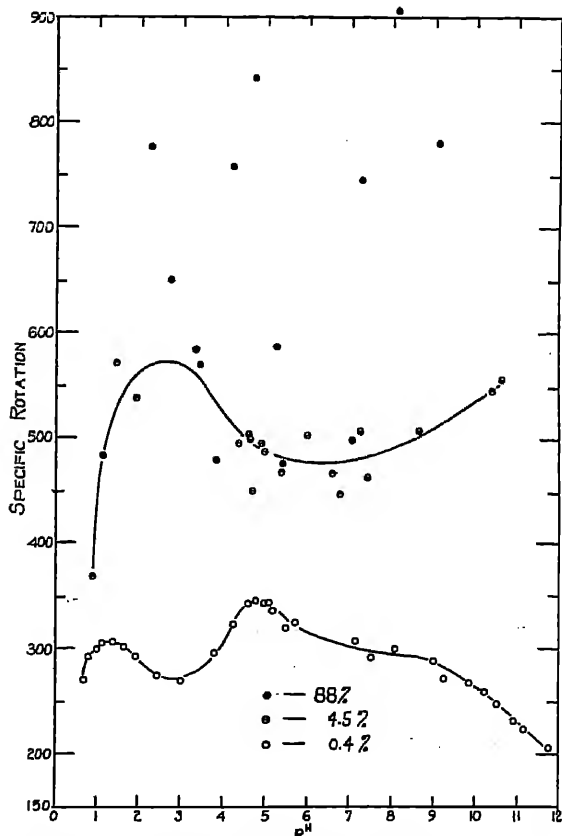


FIG. 1.—The influence of gelatin concentration upon the specific rotation of gelatin systems at 15°C.

the lower temperatures, unlike those at temperatures above 30° C., is not independent of the gelatin concentration. At this temperature the specific rotation increases with each increase in the gelatin concentration.⁵ Figure 1 shows that at a concentration of 88 per cent gelatin

⁴ Kraemer, "Colloid Symposium Monograph," Vol. 4, New York, The Chemical Catalog Co., Inc., 1926, p. 102; G. H. Joseph, Thesis, University of Wisconsin, 1927.

⁵ Kraemer and Fauselov, *J. Phys. Chem.*, 32, 894 (1928).

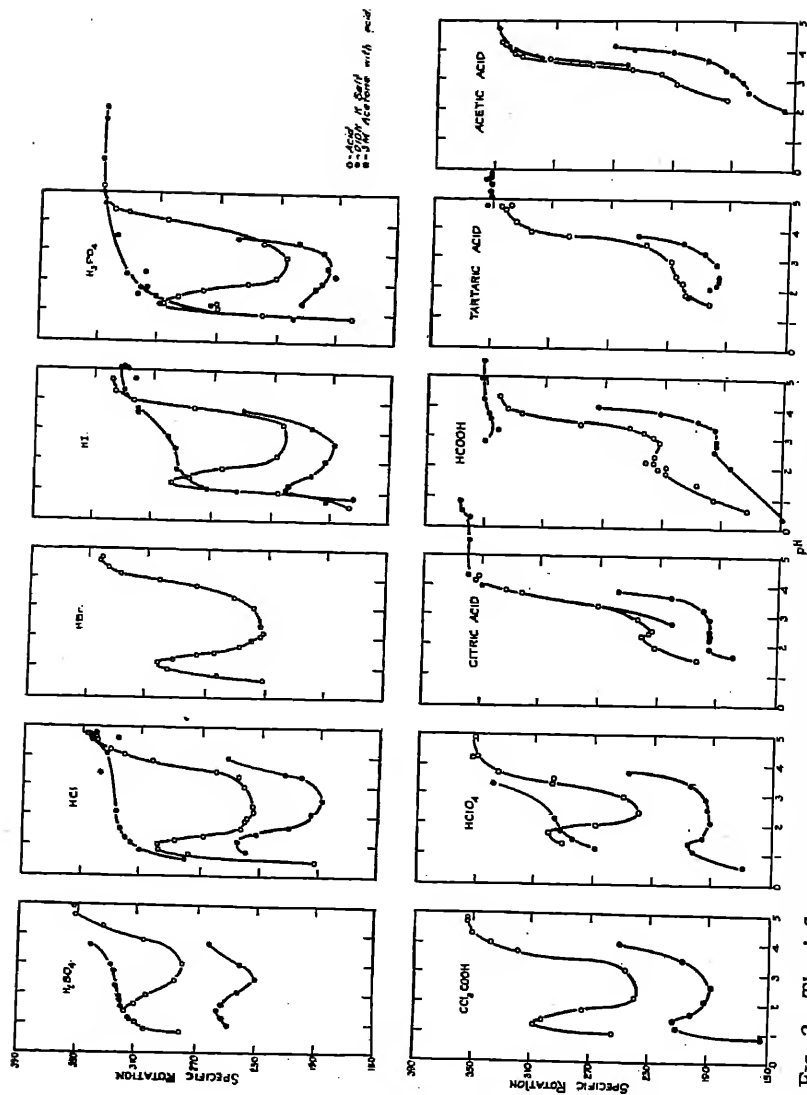


FIG. 2.—The influence of different acids and their potassium salts upon the optical activity of gelatin systems at 15°C. with hydrogen-ion activities greater than that of isoelectric gelatin.

the specific rotation is roughly two and one half times that at 0.4 per cent.⁶ The value of φ/C for the gelatin phase, with φ calculated from the same equation used in the case of the systems at 30° C., gives values which increase with each increase in the gelatin concentration until at concentrations above one per cent it has no real significance. The curve showing the variation in contraction of gelatin systems with pH as they change from the sol to the gel state⁷ does not exhibit discontinuities in the regions of maxima in relative resistance to shear and specific rotation at the lower temperatures. From these observations it is evident that the increase in specific rotation and relative resistance to shear at the lower temperatures is due to some cause other than change in the degree of dispersion or marked alteration in the degree of hydration of the gelatin. Instead it would appear that, except at the isoelectric point, the gelatin micellae at these temperatures are of about the same character as at the higher temperatures; but that they have become attached to one another by intermicellar attraction, which gives to the system the higher resistance to shear and the increase in optical activity.

THE INFLUENCE OF DIFFERENT ACIDS

When hydrochloric acid is used instead of acetic acid to control the hydrogen-ion activities of the systems the nature of the curves for specific rotation and relative resistance to shear between the isoelectric point of gelatin and pH 3 is the same as that for series with acetic acid, but at the higher hydrogen-ion activities an additional discontinuity occurs in all properties. With this acid the specific rotation and relative resistance to shear at 30° C. decrease to a minimum at a pH of about 0.7 while the Tyndall intensity exhibits a slight maximum at this value. At temperatures below 25° C. a maximum in specific rotation and relative resistance to shear appears at a pH value slightly higher than 0.7, *i.e.*, 1.2 to 1.4. The Tyndall intensity, however, does not increase appreciably except at a pH of about 0.7. This type of curve for specific rotation and relative resistance to shear appears to be characteristic of all series of systems with strong inorganic acids, but is not characteristic of series with organic acids.

The results of a series of determinations of the properties of systems with several representative acids, simultaneously brought to equilibrium at 15° C. to reduce experimental error to a minimum, show the influence of these acids upon the optical activity of gelatin systems throughout the range of hydrogen-ion activity (Fig. 2). In all of these series

⁶ The experimental error in the case of systems with 88 per cent gelatin is very large, but it cannot account for the generally higher values obtained for these systems. If the optical activity of a system is followed during the drying process the specific rotation is found to increase continuously with the removal of water.

⁷ To be published.

the curves for specific rotation and relative resistance to shear (curves for relative resistance to shear are omitted in this paper) between the isoelectric point and pH 3. are practically independent of the acid used, but in the region of high hydrogen-ion activity the specific influence of the different acids is to be observed. The magnitude of the specific rotation and relative resistance to shear of systems in the region of high acid concentrations varies somewhat with different acids, but the widest variation is in the difference between the series with strong inorganic acids and those with the organic acids. Though some of the organic acids may be made to give pH values as low as 0.5 the gelatin systems with these acids do not give rise to the increase in specific rotation and relative resistance to shear at the lower temperature in the region of pH 1.2 nor to the increase in Tyndall intensity in the region of pH 0.7. This appears to show a relationship between the appearance of the formation of the maximum in specific rotation and relative resistance to shear in systems in the region of pH of about 1.2 at low temperatures and the appearance of increased turbidity at a slightly higher acid concentration, and it shows a difference in the influence of different acids upon the properties of gelatin systems. It does not, however, show a wide variation in the influence of the different strong acids.

When one of the stabilizing factors of gelatin systems, *i.e.*, hydration, is decreased somewhat by the addition of acetone to the extent of 3 mols per liter, the specific influence of the different acids is made more evident (Fig. 2). When the systems with the acetone are simultaneously brought to equilibrium at 15° C. and their optical activities determined at this temperature, the specific rotation values are noticeably less throughout the pH range, but the reduction is not the same for the systems with the different acids. By superimposing the curves for the different acids upon one another (Fig. 3) the variation in influence is made more evident. In this series, sulfuric, hydrochloric, phosphoric, trichloroacetic, perchloric, citric, formic and acetic acid,⁸ there appears to be no relationship between the influence of the acid and the valence of the anion in question. On the contrary, it resembles very much a Hofmeister series for the precipitation of lyophobic systems.

INFLUENCE OF DECINORMAL POTASSIUM SALTS

Since the character of the curves for specific rotation and relative resistance to shear for gelatin systems between the isoelectric point and pH 3 is nearly the same for all acids it would appear that in this

⁸ The hydrogen-ion activities of systems with CCl_3COOH in this series and HgCl_2 in another series were not determined. It was assumed that the activities for systems with CCl_3COOH would be the same as those with HCl of the same concentrations, and that the addition of 0.1N HgCl_2 to systems with HCl would not change the hydrogen-ion activities to a large extent.

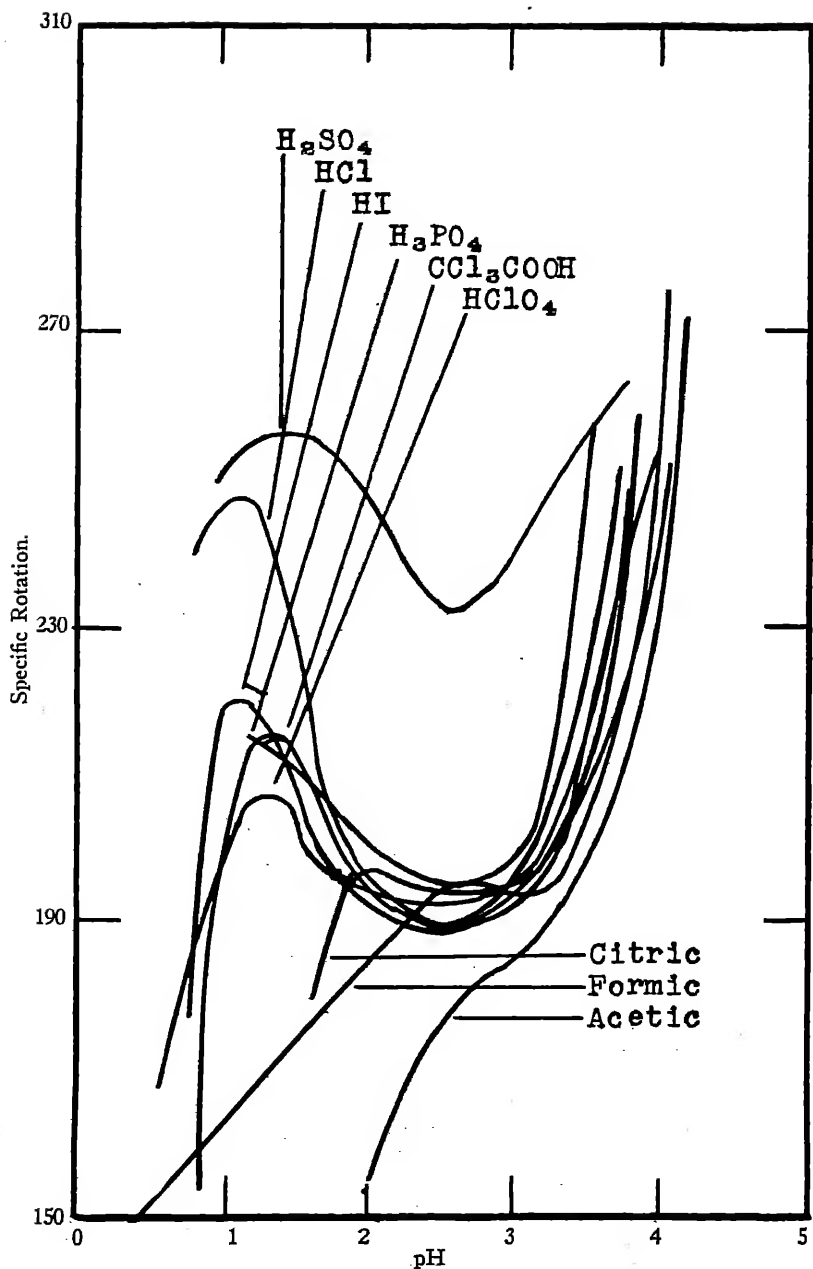


FIG. 3.—The influence of different acids upon the optical activity of gelatin systems at 15°C. in the presence of 3 M acetone.

range the hydrogen-ion activity of the system is the most important factor, but from the difference in the behavior of systems with the different acids at higher acid concentrations, however, it is evident that some factor other than hydrogen-ion activity or valence of the anion is influential. To determine whether this were due to the specific influence of the anion of the acid or some other factor the concentration of the anion was increased throughout the pH range to about the concentration as at pH 1.2 with acid alone by adding the potassium salts of the respective acids until the concentration of 1/10 equivalent per liter was reached. If the anion of the acid is the cause of the variation in behavior of the systems at the lower pH values the higher concentration of the anion caused by the addition of the salt should cause approximately the same influence upon the systems at pH 3 as that caused by the acid alone at pH 1.2. The results of this study (Fig. 2) show that in all cases the salts of those acids which gave a pronounced maximum in specific rotation and relative resistance to shear in the region of pH 1.2 caused an increase of about the same magnitude in these properties in the region of pH 3, while the potassium salts of acetic acid and citric acid, which gave no maximum in the region of pH 1.2, caused no increase in properties at pH 3.⁹ This, in the opinion of the author, confirms the view that the anions of different acids have a specific influence upon the optical activity and relative resistance to shear of gelatin systems in the region of pH 0.5 to 3, and that the cause of the variation in properties of these systems cannot be attributed to the hydrogen-ion activity of the system nor to the valence of the anion.

EFFECT OF DIFFERENT CATIONS ON THE ACID SIDE OF THE ISOELECTRIC POINT

Since the character of the curves for relative resistance to shear and specific rotation in the region between pH 3 and the isoelectric point of gelatin seemed to be independent of the acid used to control the pH values of the systems, the influence of different amounts of acids upon the properties of these systems was attributed to the hydrogen ion. To determine whether the presence of cations other than the hydrogen ion would cause any variation in the behavior of systems on the acid side of the isoelectric point, a comparison was made of the influence of the chlorides of sodium, calcium, zinc and bivalent mercury, and the influence of the sulphates of ammonium, sodium, magnesium and aluminum upon the series throughout the pH range with the respective acids. It may be noted in Figure 4 that though there is a small varia-

⁹ A miscalculation in the amount of formic acid necessary to give systems with low pH values in the presence of potassium formate caused this series to be incomplete, and time would not permit further work with the series. It is impossible, of course, to carry the study with 1/10 potassium tartrate to the higher acid concentrations.

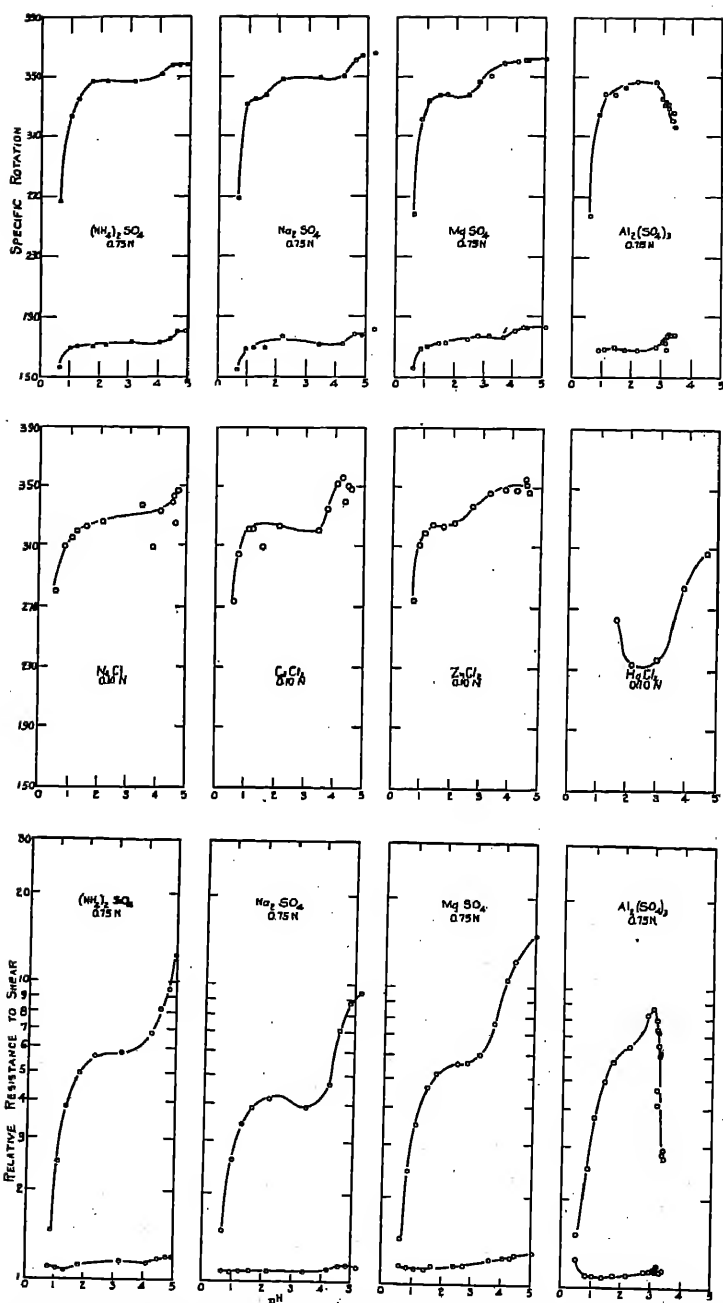


FIG. 4.—The influence of different cations upon the optical activity and relative resistance to shear of gelatin systems at 15°C. and 30°C. with hydrogen-ion activities greater than that of isoelectric gelatin.

tion in the character of the curve in the region of pH 0.5 to 3 it is not influenced to a large extent by the different cations, but in the region between pH 4 and the isoelectric point of the gelatin there is in some cases an appreciable variation in the character of the curves for the

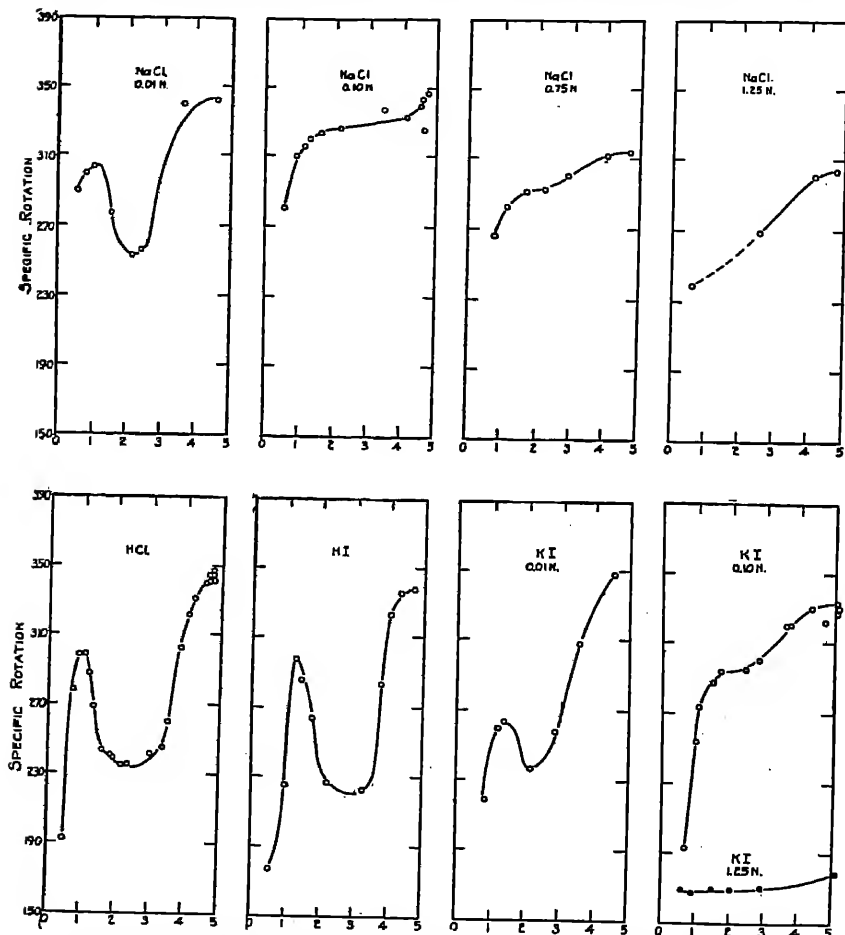


FIG. 5.—The influence of varying amounts of NaCl and KI upon the optical activity of gelatin systems at 15°C.

different salts of the same acid. To attribute this variation to chemical combination of the gelatin in this pH range with the cation of the salt is not in conformity with the view that gelatin acts as a base in systems with hydrogen-ion activities greater than that at the isoelectric point.

On the contrary, it shows the difference in the stabilizing action of different salts upon isoelectric gelatin, while failure of the different cations to alter the curve between pH 0.5 and 3 is in conformity with the view that the variation of the curves for systems with different acids at pH values below 3 is due almost entirely to the anion.

EFFECT OF VARYING AMOUNTS OF SALTS

To determine the influence of varying amounts of different salts upon the properties of gelatin systems at definite hydrogen-ion activities,

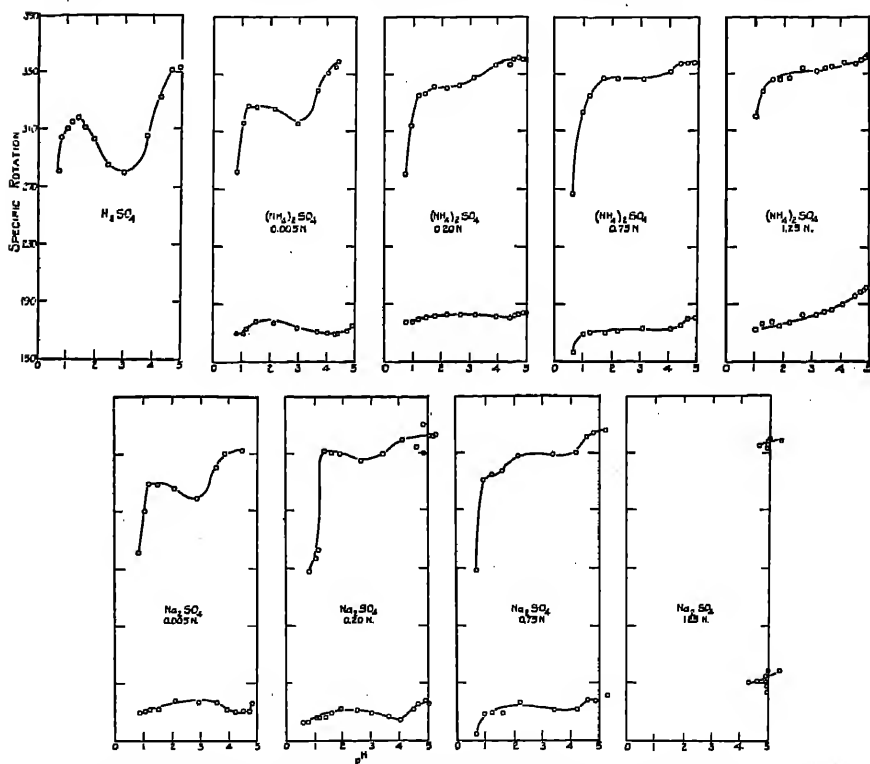


FIG. 6.—The influence of varying amounts of Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ upon the optical activity of gelatin systems at 15°C. and 30°C.

series of systems with potassium iodide in hydriodic acid, sodium chloride in hydrochloric acid, and sodium and ammonium sulfates in sulfuric acid were prepared. In each of the four series (Figs. 5, 6, and 7) it may be noted that at 15° C. the addition of a small amount of salt

increases the specific rotation and relative resistance to shear throughout the pH range from 1.2 to the isoelectric point, but not at pH values below 1.2. Further addition of salt increases both properties to a maximum and then causes a decrease. The amount of salt required to carry the change in properties to a maximum varies somewhat with the salt

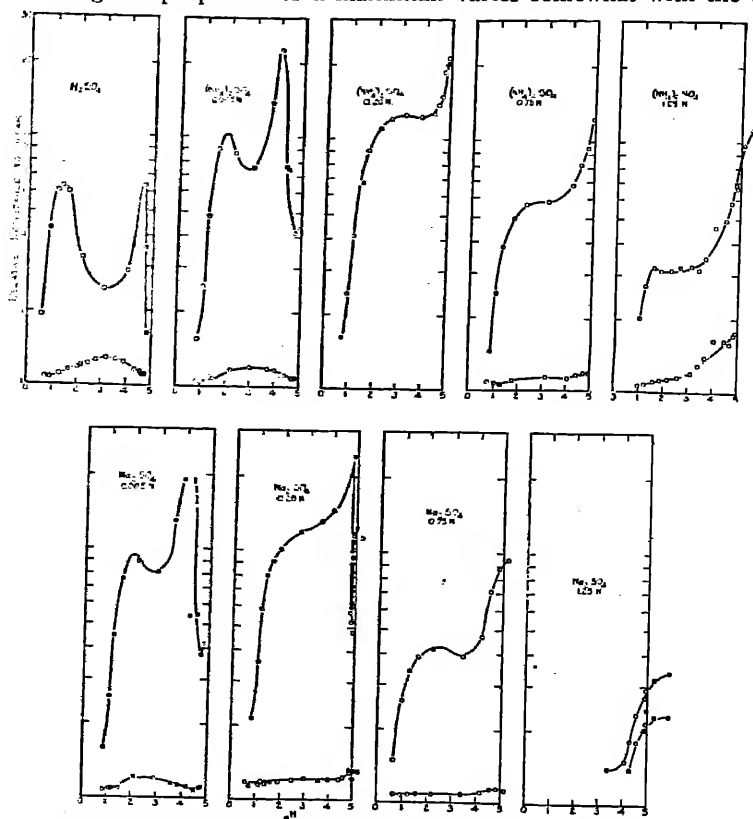


FIG. 7.—The influence of varying amounts of Na_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$ upon the relative resistance to shear of gelatin systems at 15°C . and 30°C .

and the hydrogen-ion activity, but the point of maximum is reached in the two properties with approximately the same concentration of anion as that which gives the maximum with acid alone at pH 1.2. In all cases except systems with potassium iodide and calcium chloride the formation of a noticeable turbidity occurs at the higher salt concentrations, just as at pH 0.7 with acids alone. This indicates that the addition

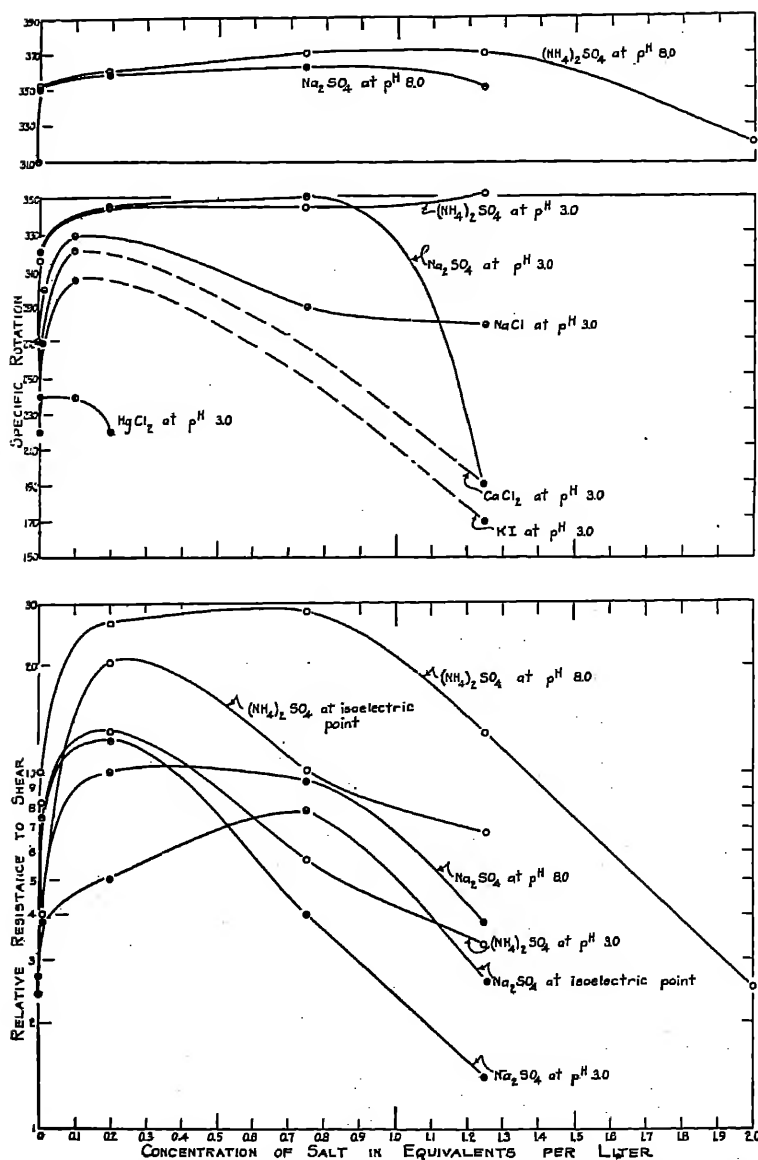


FIG. 8.—The influence at 15° of varying amounts of different salts upon the optical activity and relative resistance to shear of gelatin systems at definite hydrogen-ion activities.

of a certain concentration of anion, whether added in the form of salt or acid, has the same influence upon the properties of gelatin systems.

A comparison of the influence of different concentrations of salts upon the properties of systems at 15° C. at definite pH values, *i.e.*, 3, 4.9, and 8 (Fig. 8) gives curves which are quite similar to those showing the influence of different amounts of salts upon the electrokinetic potential of lyophobic systems. This suggests that the influence of salts upon gelatin systems is similar to that of electrolytes upon the lyophobic systems.

INFLUENCE OF NON-ELECTROLYTES AND TANNING AGENTS

To determine whether the above described behavior of the systems in all regions, other than at the isoelectric point, could be attributed to dehydration or a change in the nature of the gelatin micellae, or whether it must be attributed to electrolyte influence, the specific rotation and relative resistance to shear of gelatin systems were determined with varying amounts of non-electrolytes and tanning agents at representative pH values, *i.e.*, 1.4, 3.0, 4.2, 4.7, and 8.7. The concentration of these reagents—acetone, ethyl alcohol, glycerol, urea, formaldehyde, phenol, pyrogalllic acid, and tannic acid—ranged from 0.0001*M*, in the case of the tanning agents, and 0.005*M*, in the case of the others, to the highest concentrations which would permit the formation of transparent systems. The results of these series of determinations may be published in detail elsewhere, but it is sufficient to state in this report that all of these substances except glycerol caused a decrease in the specific rotation and relative resistance to shear of gelatin systems at 15° C. Glycerol caused very little change in the optical activity or relative resistance to shear for concentrations as high as 9 *M*.¹⁰ This, it seems, shows that the increase in relative resistance to shear and optical activity of gelatin systems at 15° C. by the addition of small amounts of electrolytes cannot be attributed to a dehydrating action or change in the nature of the gelatin micella surface, but that it is due to the same mechanism as the influence of electrolytes upon the various properties of lyophobic systems.

MECHANISM OF THE SOL-GEL TRANSFORMATION

The temptation to enter the realm of speculation concerning the mechanism of the change from the sol to the gel state in the case of gelatin systems is too great to resist. Since the increase in gel formation

¹⁰ In some cases there was a very slight increase in both properties with the addition of very small amounts of the above reagents, but these variations were of the order of magnitude of the experimental error and may possibly be attributed to that; but in no case were they similar in any respect to the behavior caused by the addition of electrolytes.

at the lower temperatures is so markedly influenced by the addition of electrolytes one is inclined to think of the influence of these substances upon the electrokinetic potential of the gelatin micellae. It is unfortunate that this has not been determined over a wider range of hydrogen-ion activity and salt concentration. However, over the range that has been studied by Morioka and by Okayama¹¹ it may be noted that a minimum in specific rotation and relative resistance to shear occurs in gelatin systems at 30° C. wherever there is a low electrokinetic potential, while at 15° C., except where the electrokinetic potential is zero or very nearly so, a high specific rotation and relative resistance to shear appears in these regions.

At the higher electrolyte concentrations it is in conformity with the behavior of high concentrations of electrolytes upon heterogeneous systems to believe that the electrokinetic potential of the gelatin micellae would be decreased either by the addition of acids to give pH values lower than 3 or by the addition of equivalent concentrations of salts. The fact that the addition of high concentrations of strong inorganic acids or most of their salts causes a turbidity or a coagulation of the gelatin systems seems to lend support to this view. It is likely that at pH 0.7 with the strong inorganic acids or with equivalent concentrations of their salts the potential is reduced to zero. Then, since the maxima in resistance to shear appear in regions adjacent to, but not coinciding with, the regions of coagulation and in the region of pH of about 8, it appears that low electrokinetic potential on the gelatin micellae permits them to be more strongly attracted to one another than they are in systems in which the electrokinetic potential is higher. This attraction between the micellae, together with the fact that they have not lost much of their attraction for the dispersion medium, gives to the systems the higher resistance to shear and the accompanying increase in optical activity. Hence these regions of maxima in properties at low temperatures occur at a pH of about 1.2, 4.5, 5.3, and 8.

The complete removal of the electrokinetic potential is also associated with a decrease in the affinity of the gelatin for the dispersion medium. This gives rise to the formation of the high degree of turbidity or even coagulation of the gelatin phase at the isoelectric point of electrolyte free gelatin, at a pH of about 0.7 with strong inorganic acids and in the systems with high concentrations of salts of the strong inorganic acids.

SUMMARY

In summary it may be stated that the variation in properties of gelatin systems at low temperatures as described in this and other similar

¹¹ Bancroft, "Applied Colloid Chemistry," Second Edition, New York, McGraw Hill Book Co., 1926, p. 342.

studies has not been accounted for by the view that the gelatin combines stoichiometrically with electrolytes. Experimental evidence seems to the author to indicate that the influence of electrolytes upon the optical activity and relative resistance to shear of gelatin systems is the same as the influence of these substances upon the properties of lyophobic systems, but that the evidence of the influence is complicated somewhat by the amino acid nature of the micellae surfaces and is masked to a certain extent by the increased stability of these systems caused by the high affinity of the gelatin phase for the dispersion medium. However, these complications do not alter the view that the behavior of gelatin systems can better be explained by the laws obtained from the behavior of highly dispersed heterogeneous systems than by the classical laws of stoichiometry.

*University of Wisconsin,
Madison, Wisconsin.*

INFLUENCE OF GEL STRUCTURE UPON THE TECHNOLOGY OF SMOKELESS POWDER MANUFACTURE*

BY FRED OLSEN

The function of a weapon, whether it be a cannon or a small arms piece, is to cause a projectile to hit a target. The force necessary to propel the missile is generated by a propellant powder. Most of the propellants at the present date comprise charges of gelatinized nitrocellulose in the form of grains whose dimensions depend upon the caliber and power of the gun in which the powder is used.

For many years it has been the practice to control the chemical composition of these grains within very narrow limits. However, in spite of the greatest care being taken to insure uniformity, there has always been dissatisfaction on the part of artillerymen with reference to failures to hit the target, even though all the corrections, such as change in elevation or traverse of the piece, allowance for wind, etc., were applied. The powder is usually, and sometimes justly, blamed for the target being missed.

Powder technologists have observed that considerable variations occur in the dimensions of the grains of powder even though care is exercised in controlling the dimensions of the dies through which the powder is extruded. The differences encountered in the dimensions of the grain are now attributable mainly to variations in the degree of shrinkage, which occurs when the volatile solvents employed in the gelatinization of the nitrocellulose are being removed. Figure 1 shows

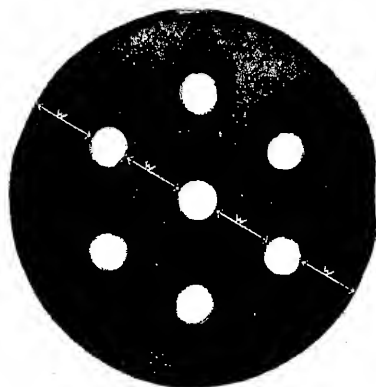


FIG. 1.—Cross Section of Grain of Powder. Dimension W is the Web of the Powder.

a cross section of a grain of powder containing seven perforations arranged symmetrically about the longitudinal axis of the grain. The distances marked " W " are known as the web of the powder; and since powder has been found to burn in layers parallel to the surface, it will

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FIG. 2.—Nitrocellulose Fibers from Cotton Linters, Seen by Transmitted Light.
X 100.

be seen that the web is the critical dimension of the grain. Powder which is used in the famous war-time weapon, the French 75-mm. gun, is required to have a web of about 0.020 inch. Careful ballistic tests have indicated that a variation in web thickness of one one-thousandth of an inch will cause the projectile to miss the target at maximum range by 75 yards. Numerous examinations of accepted lots of powder have shown that the normal maximum variation in the web dimension of individual grains from a given lot of powder is ten one-thousandths of an inch, so that if charges were made for the gun in which these maximum differences prevailed in each of the charges, a variation in range of 750 yards would result. The remedy which is generally applied consists in blending the grains of powder, and the known accuracy of the weapons is undoubtedly in part attributable to this practice of mixing heavy- and light-web grains. However, this remedy is only partially satisfactory to powder technologists, who are now spending much effort in eliminating, if possible, those factors which cause variations in the shrinkage of the gel.

It has been found that when uniform shrinkage of the powder grain has occurred, the structure of the gel is correspondingly uniform. It is therefore the problem of the powder maker to convert fibrous nitrocellulose into as homogeneous a gel as possible.

Gelatinization of nitrocellulose depends upon several factors, among the most significant of which, under present manufacturing conditions, are the chemical constitutions of the nitrocellulose and solvents, the physical conditions of the fibre, and also the methods of processing.

Nitrocellulose having a nitrogen content of more than 13 per cent is not readily gelatinized by the common solvents, whereas nitrocellulose whose nitrogen content is about 12 per cent is very readily gelatinized.

Figure 2 shows a normal sample of nitrocellulose closely resembling the cotton-linter fibres from which it was made. When examined under polarized light (Fig. 3), the lack of uniformity in nitration is apparent in that the beam of polarized light has been variously turned by the different strands of nitrocellulose. This variation in nitrogen content materially contributes to the inhomogeneities of the gel.

The action of solvents upon nitrocellulose is first a swelling of the fibre as is shown in Figures 4A and 4B, which illustrate nitrocellulose before and after exposure to solvent vapors.

The subsequent action of the solvent consists in the dispersion of the gelatinized nitrocellulose, and many of the steps in processing powder are designed to give as complete a degree of dispersion as possible. The first of these steps is in a mixer of the dough-kneader type where nitrocellulose, ether, and alcohol are incorporated. The nitrocellulose exhibits a tendency to ball, and when a section of the



FIG. 3.—Same Specimen of Nitrocellulose as in Figure 2, Viewed with Polarized Light.
X 100.



FIG. 4A.—Nitrocellulose Fibers Before Action of Solvent. $\times 150$.

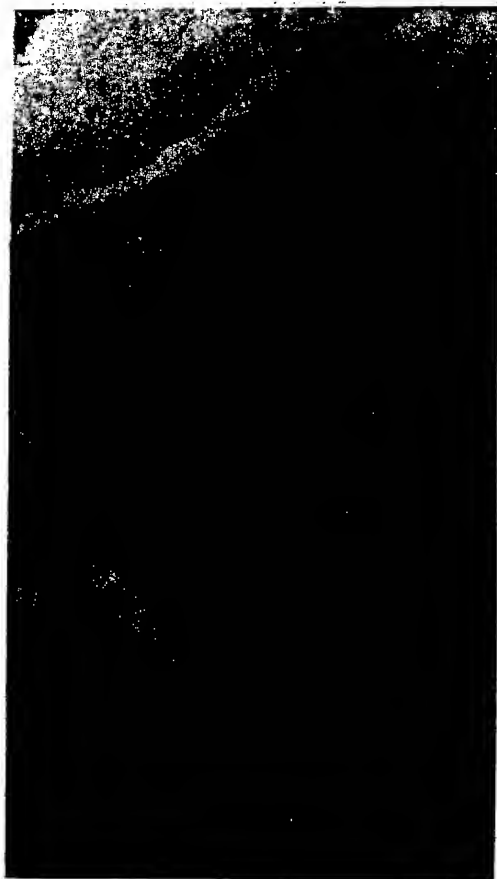


Fig. 4B.—Swollen Nitrocellulose After Action of Solvent. $\times 150$.

consolidated charge from the mixer is examined (Fig. 5A) these balls of nitrocellulose appear as partially gelatinized nodular masses. A further dispersion of these is effected by the kneading action afforded

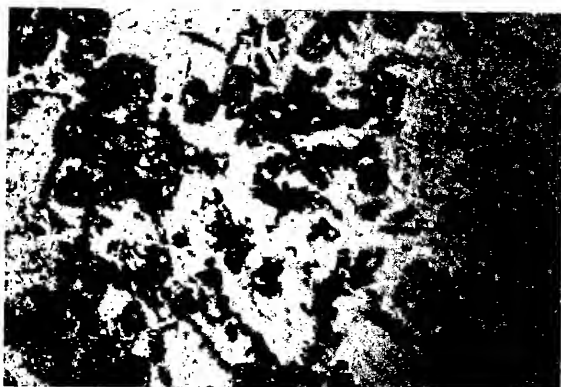


FIG. 5A.—Section of Consolidated Charge from Mixer. $\times 100$.

by extruding the nitrocellulose gel through a series of screens and perforated metal plates in the form of strands known as macaroni. These strands are consolidated, and a section from the block (Fig. 5B)

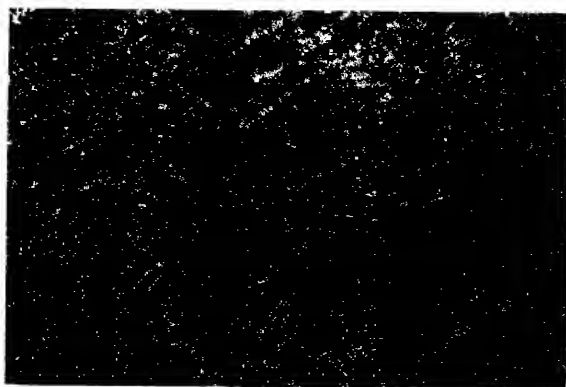


FIG. 5B.—Section of Consolidated Charge from Macaroni Press.
 $\times 100$.

shows a marked increase in the uniformity of the gel. The globular masses have been elongated into a fibrous form, the longer axis indicating the direction of extrusion.

This blocked charge is now extruded in a graining press through



Fig. 6.—Sections of Powder Grains Containing Various Percentages of a Plasticizer. $\times 65$.

dies from which a multi-perforated strand is fed to a machine which cuts the strand into short grains.

By increasing the amount of solvent used, and also by modifying the solvent composition, improvements in homogeneity can be secured. The powder technologist has borrowed from the lacquer manufacturer the use of plasticizers by means of which a greater dispersion of the gelled particles is often obtained.

Figure 6 shows four sections through powder grains in which varying percentages of a plasticizing agent have been used. The first section is of a normal powder grain; the black streaks indicate the imperfectly gelatinized nitrocellulose, since these particles restrict the passage of the light beam passing through the section. The second section shows that the addition of 2 per cent of the plasticizer has had practically no effect upon the gel structure. The addition of 5 per cent of the plasticizer, however, has not only reduced the number and extent of the incompletely gelatinized particles, but has also made it possible for the solvent to diffuse more readily through the grain, since the time of drying has been reduced from about 150 days in the case of the first two sections to about 40 days. The last section shows the marked increase in homogeneity which is brought about by adding 7 per cent plasticizer. The time of drying in this case is about 25 days. The addition of more plasticizer produces no additional advantage either as regards uniformity of gel or time of drying.

Powders comprising a uniformly gelatinized nitrocellulose have been found to have a variation in velocity as small as 4 feet per second in contrast with 50 feet per second variation with powders of a less degree of homogeneity.

Although much can be done to secure greater homogeneity by improving the technique of various steps in the gelatinization process, some of the difficulties are inherent to the type of cellulose employed. The cotton-linter hair is a relatively heavy walled tube, closed at one end, and penetration of this tube by the nitrating acids, by the purification liquors, and subsequently by the solvents employed in gelatinizing nitrocellulose, is quite difficult to accomplish. A further difficulty towards obtaining a homogeneous gel is imposed by the knotted form in which the cotton linters occur. Figure 7 shows two normal samples of cotton linters in which the clumps of cotton are evident. These clumps offer a still greater resistance to uniform penetration by the nitrating acids, and constitute one of the main contributing causes to the variation in degrees of nitration from the outside to the inside of the clump, and hence of later variations in the degree of gelatinization.

The central portion of the picture shows a sheet of tissue compris-

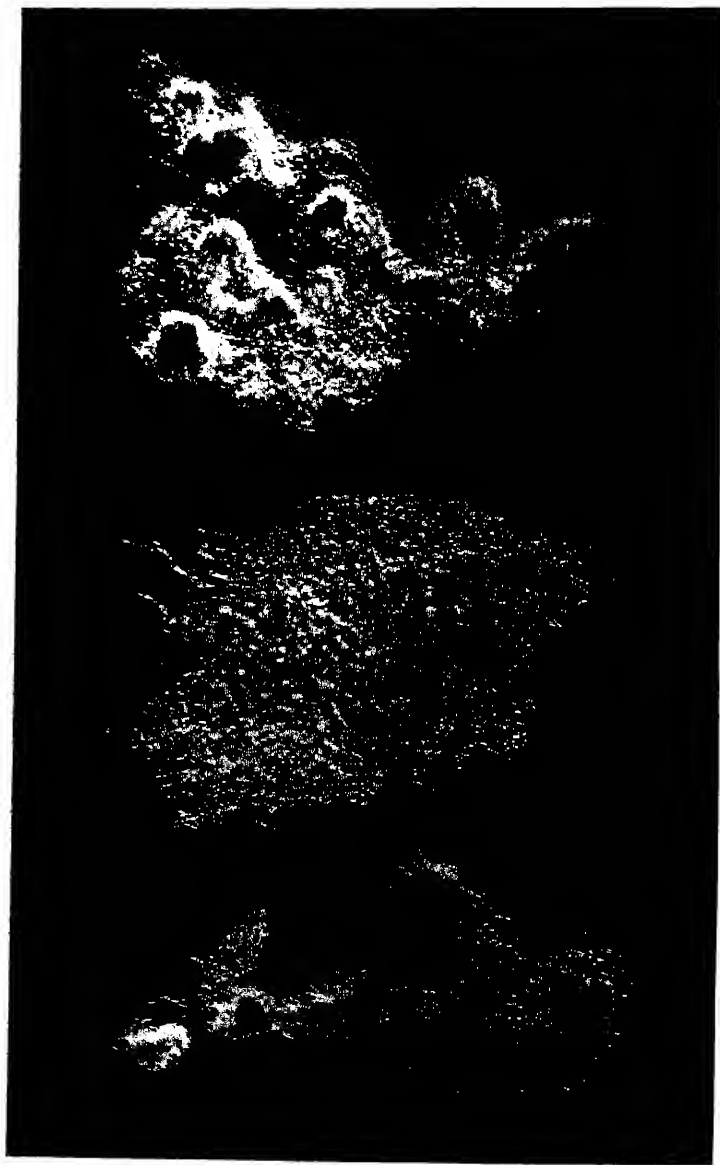


FIG. 7.—Two samples cotton linters with central sample of Spruce sulfite tissue, showing knotted masses or neps in linters, as contrasted with even texture of the wood pulp. $\times 3\frac{1}{2}$.



FIG. 8A.—Beaten Fibre of Nitrocellulose. $\times 150$.



FIG. 8B.—Unbeaten Fibres of Nitrocellulose. $\times 150$.

ing spruce sulfite pulp, the uniformity of texture of which is quite marked.

The knotted condition of the fibres also hinders the purification process in that it makes the removal of the acid occluded by the fibres more difficult. Partially to avoid this condition the practice of beating or grinding the nitrocellulose has been adopted. The ideal condition, of the fibre is where every fibre has been completely opened or flattened; Figure 8A shows this condition recently achieved in studies on improvements in beating. This condition of the fibre also lends itself to uniform and rapid gelatinization. Figure 8B shows an unbeaten fibre at the same magnification; not only is the heavy-walled character of the cotton hair clearly seen, but also the increase in surface exposed by the action of the beater is shown by the contrast between the beaten and unbeaten fibers.

*Picatinny Arsenal,
Dover, New Jersey.*

GRAIN GROWTH IN SILVER HALIDE PRECIPITATES*

BY S. E. SHEPPARD AND R. H. LAMBERT

INTRODUCTION

A previous paper¹ instanced different types of the change of dispersity, or grain growth, of silver halide precipitates. In particular, some attempt was made to distinguish the part played by flocculation in this process.

In the following paper are given particulars of an experimental study of the grain growth of silver bromide precipitates which show that the process is regulated by definite quantitative laws. It is not claimed that the full ambit of these laws has been mapped out² but it is believed that their outline has been discerned with promise of wider bearing.

The production of a precipitate, *i.e.*, the formation of a new solid phase, is broadly divisible into two periods or moments.³ The first consists in the inception or nucleation of the new phase; the second in the growth of grains, either by the *accretion* from within or by aggregation of particles. Although these periods are ideally separable, in practice it is not always possible or desirable to separate them. The fundamental investigations of von Weimarn⁴ have shown that with practically every substance produced at sufficiently low solubility, the second moment may be so reduced or delayed that jellies of the substance can be observed. In many cases, the primary sols are coagulated to gels (flocculated) in which the number of primary particles is the same as that of the sols.⁵

There exists a group of systems⁶ in which the solubilities are sufficiently higher than in those just mentioned to permit aggregation to microscopic dispersions within a reasonable period of time. Among these, the silver halides found in aqueous solutions are notable, and

* Communication No. 357 from the Kodak Research Laboratories.

¹ Sheppard and Lambert, "Flocculation and Deflocculation of the Silver Halides," Colloid Symposium Monograph, Vol. 4, Chemical Catalog Co., Inc., New York, 1926, p. 281.

² Von Weimarn, "The Precipitation Laws," *Chem. Rev.*, 2, 217 (1925).

³ To avoid confusion these terms are used instead of *phase* as a historical condition.

⁴ Alexander, "Colloid Chemistry," Vol. 1, Chemical Catalog Co., Inc., New York, 1926, p. 27.

⁵ Odén, "Sedimentation Analysis and Its Application to the Physical Chemistry of Clays and Precipitates," in Alexander's "Colloid Chemistry," Vol. 1, Chemical Catalog Co., Inc., New York, 1926, p. 861.

⁶ *System* is used to indicate a substance and its thermodynamic environment.

of practical importance because these microcrystalline dispersions when found in gelatin are the photographic emulsions. The following experimental procedure enables us, not to secure the inhibited jelly or the flocculated-out stages, but what we may term primary, reproducible, microcrystalline dispersions which could then by appropriate change of the solubility be carried on in grain growth.

EXPERIMENTAL PROCEDURE

A stock solution of potassium bromide was made up from purified material, recrystallized by us three times. Silver nitrate was found sufficiently pure as obtained to be regarded as satisfactory in preparing a stock solution.

The apparatus used for precipitation is shown in Figure 1. Two glass bulbs of 500 cc. capacity have a common access to the manometer and air pressure. An air escape at *C* removes large fluctuations in pressure. The liquids in *A* and *B* on exerting pressure meet at the

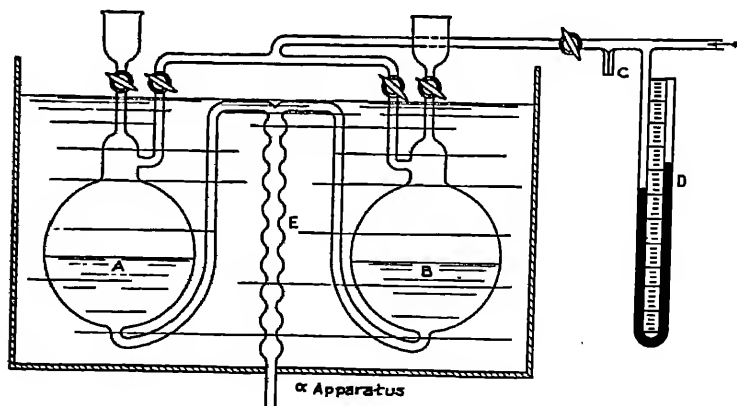


FIG. 1.

upper end of *E*. This type of tube out of a great many types studied was found to give the most efficient mode of mixing. Mixing was complete after the second bulb had been passed by the material. Stopcocks were inserted at appropriate places for filling the bulbs, washing the apparatus and equalizing rates of flow. The time of mixing was noted and was reproducible to a point where this factor does not contain the controlling error. This was verified by making two runs of widely different times of total mixing and results showed little difference on the resulting size-distribution curves.

After precipitation, the material was placed in a vessel, in the same thermostat as that containing the precipitating apparatus, which had a potassium bromide solution of known concentration and volume.

Within limits the rate of stirring was found to have little influence on the growth of grains. Stirring was accomplished with a small paddle stirrer at 1200 r.p.m. Samples were removed at definite intervals of time and added to an ice-cold, very dilute solution of silver nitrate in order to completely neutralize the excess potassium bromide and thus stop growth. The silver bromide particles thus formed were far below the resolving power of the microscope thus causing no error in grain measurements.

The samples were now added to a "plating" solution of such volume that one cc. of the mixture contained the same number of molecules for each sample when spread out in a one-grain layer on a 2 by 5 inch standard plate glass, *i.e.*, 0.000004 mol. of silver bromide. This "plating" solution also contained formaldehyde, grain alcohol, saponin and gelatin in order to get good dispersion of the grains.

The plates were chilled and then dried at a temperature of 25° C., drying occupying about two hours. All work was done in the light, ripening, however, taking place in dim daylight.

Photomicrographs at 2500 diameters were made by Mr. A. P. H. Trivelli and the number of fields photographed depended on the average size of grains. They ranged from three to nine fields per plate. The prints were enlarged to 10000x so that 1 cm. on the plate corresponds to 1 μ in the photographic field. The size-frequency determination is well known so that only reference to the literature need be made.⁷

THE EXPERIMENTS

Two hundred cc. of 1.01 *N* potassium bromide and the same volume of 1.00 *N* silver nitrate each containing 0.625 per cent ash-free gelatin were placed in the two bulbs shown in Figure 1. All experiments were carried out at 60° \pm 0.5° C. The time of mixing was fifty seconds, accurate to about two seconds. The mixed material was added to 100 cc. of potassium bromide solution. The concentration of potassium bromide during ripening was 0.02, 0.04, 0.06 and 0.08 *N* in the four series of experiments carried out. The system contained 0.5 per cent gelatin in each case. Samples were removed at 30, 60, 90, 150, 240, 345, 495, 870, 1110, and 1395 minutes after ripening started.

The checks were made for each series and since for times 870 and 1110 minutes it was inconvenient to remove samples, three separate

⁷ Cf. Sheppard and Lambert, "Report of Sub-committee on Size and Shape of Sub-sieve Sizes," *A. S. T. M.* (1928); Grundlagen, "Negativverfahren," Lüppo-Cramer, Eder's Handbuch, 1927, Sheppard and Trivelli, p. 735.

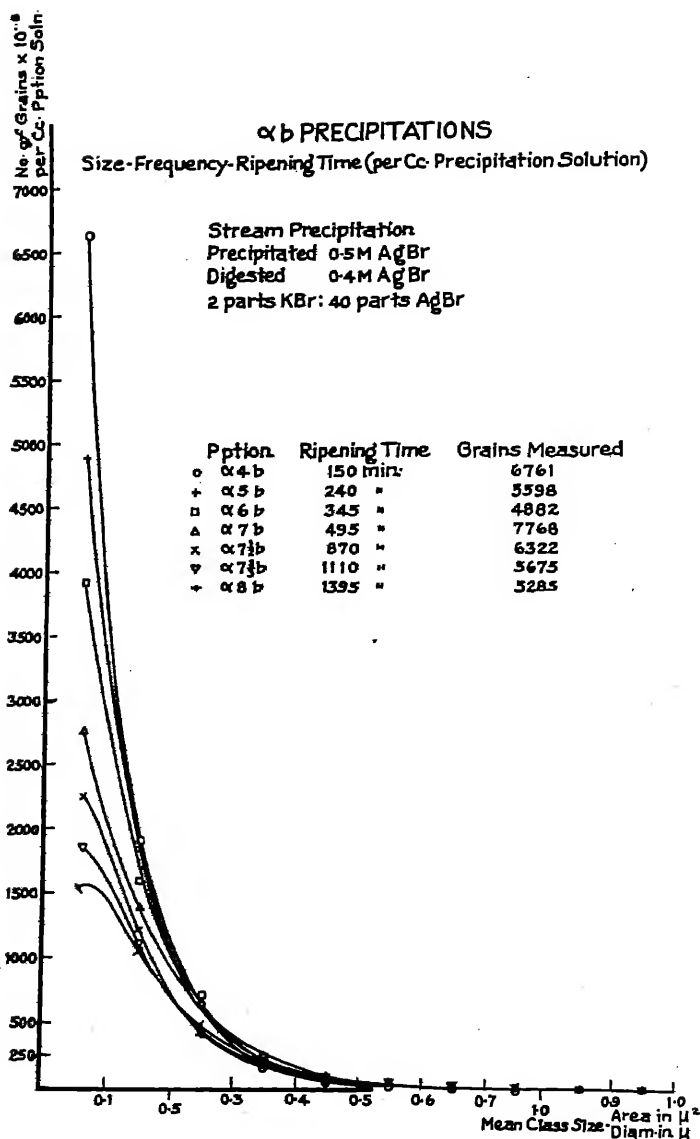


FIG. 2.

runs were made for these two samples. The fact that the data for the latter fit smoothly in the sequence of the first runs adds further strength

to the reliability of results. Nearly one-half million grains were counted and measured for the following study of the data.

EXPOSITION OF DATA

A survey of one set of data, *i.e.*, that for growth of silver bromide in 0.02 *N* potassium bromide in which the molal ratio of AgBr : KBr

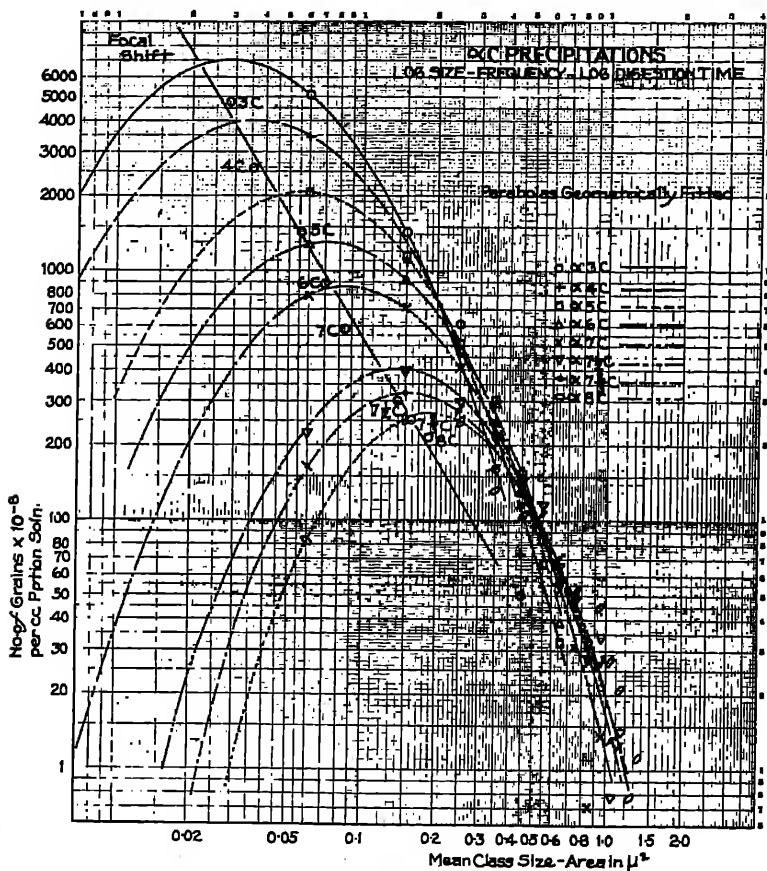


FIG. 3.

is 40 : 2 (Table I), shows that several methods of representation may be used. For a given time of digestion there is a definite size-frequency distribution. Plotting each curve for the various times of digestion on one sheet of graph paper, one obtains results as shown in Figure 2.

TABLE I. *Size-Frequency Distribution of Silver Bromide Precipitations with Time of Digestion.*
 Number of Grains $\times 10^{-3}$ per cc. of Solution as Precipitated.
 ab: 2 parts KBr: 40 parts AgBr on Digestion.

Pre- cipitation	Minutes Digestion	1	2	3	4	5	6	7	8	9	10
		0.06 μ^2	0.15 μ^2	0.25 μ^2	0.35 μ^2	0.45 μ^2	0.55 μ^2	0.65 μ^2	0.75 μ^2	0.85 μ^2	0.95 μ^2
a4b	150	6629.8	1911.8	641.6	152.7	37.0	14.3	1.2	2.4
a5b	240	4870.6	1860.6	681.0	178.9	37.0	16.7	6.0
a6b	345	3971.6	1591.0	718.0	230.2	64.4	25.1	9.6	4.8	0	1.2
a7b	495	2757.3	1380.5	647.6	239.7	90.0	44.7	10.1	4.8	3.6	2.4
a7½b	870	2250.4	1207.6	421.0	169.4	75.1	45.9	19.7	11.3	5.4	6.0
a7¾b	1110	1850.1	1116.9	416.3	173.5	74.6	47.7	22.1	16.1	9.0	9.0
a8b	1395	1528.8	1046.0	477.1	179.5	76.9	58.5	20.3	20.3	9.0	11.3

Pre- cipitation	Minutes Digestion	11	12	13	14	15	16	17	18	19	20
		1.05 μ^2	1.15 μ^2	1.25 μ^2	1.35 μ^2	1.45 μ^2	1.55 μ^2	1.65 μ^2	1.75 μ^2	1.85 μ^2	1.95 μ^2
a6b	345	0	0	0	1.2
a7b	495	0.6	1.2	1.20
a7½b	870	3.6	2.4	1.2	0.6	0	0.6
a7¾b	1110	4.2	6.6	3.0	3.0	0	1.2	0	0.6	0	0.6
a8b	1395	4.2	11.9	3.0	6.0	0.6	1.2	1.2	0.6	0	1.2

A statistical examination⁸ indicates that the curves can be represented by a modified Gaussian equation with a logarithmic exponent. If the data are, therefore, plotted on double logarithmic paper a parabola results as shown in Figure 3. The parabolas are found invariant as to shape for the entire set of experiments, they merely shift in such a manner that their foci lie on an inclined straight line.

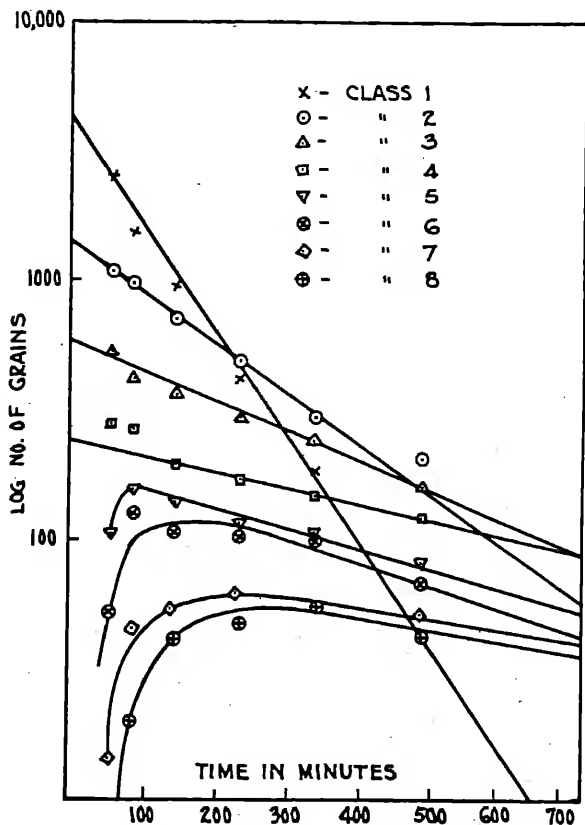


FIG. 4.

Before advancing to any theory of the total statistical distribution, it will be found advantageous to consider the change of a given class-size with time of digestion and other factors. If the logarithm of the number of grains of such a class-size be plotted against time of ripening

⁸Loveland and Trivelli, "Mathematical Methods of Frequency Analysis of Size of Particles," *J. Frank. Inst.*, 204, 193-217, 377-389 (1927).

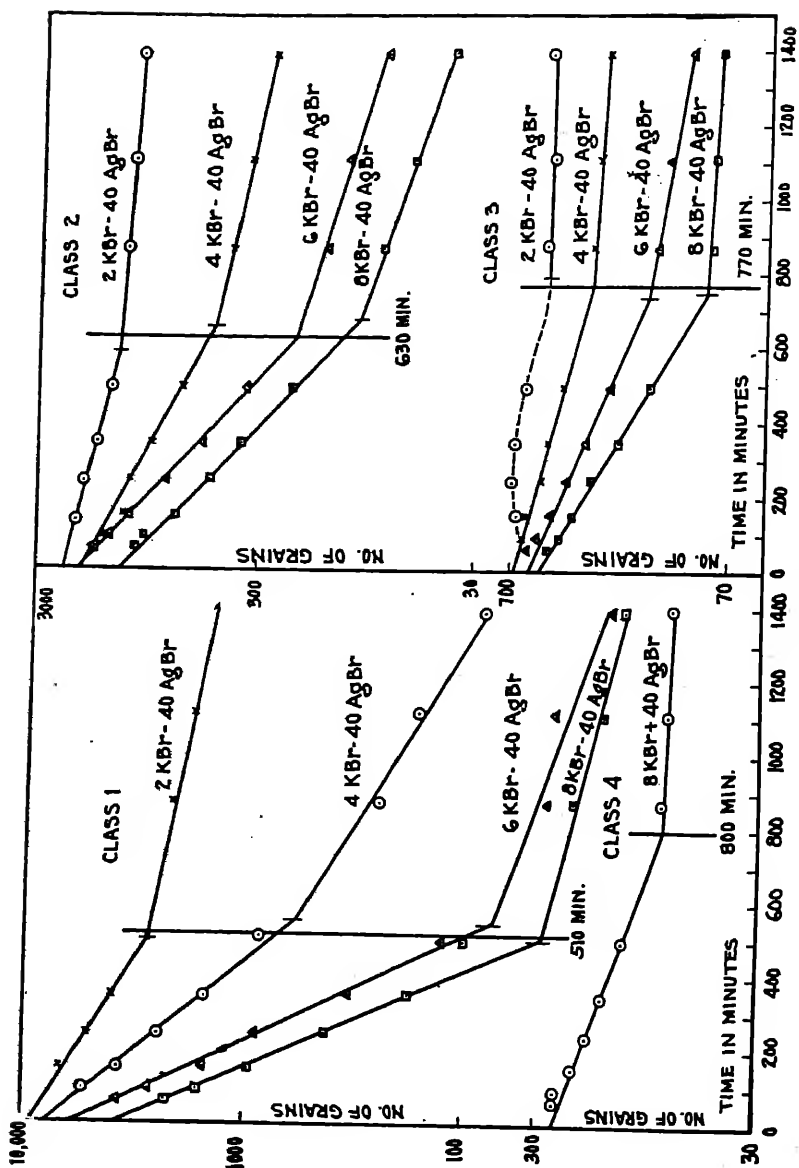


FIG. 5.

for the first four classes, straight lines result, the slopes of which are dependent on the potassium bromide concentration (cf. Fig. 4). Furthermore, it may be observed (Fig. 5) that these lines are in reality broken into two segments of widely different slopes. The break in slope is found to be independent of potassium bromide concentration, within experimental limits of error, but varies with the mean diameter of the particles almost linearly, as shown in Table II.

TABLE II.

Mean Diameter in μ	Time of Break in Min.
0.13.....	510
0.215.....	630
0.281.....	770
0.333.....	800

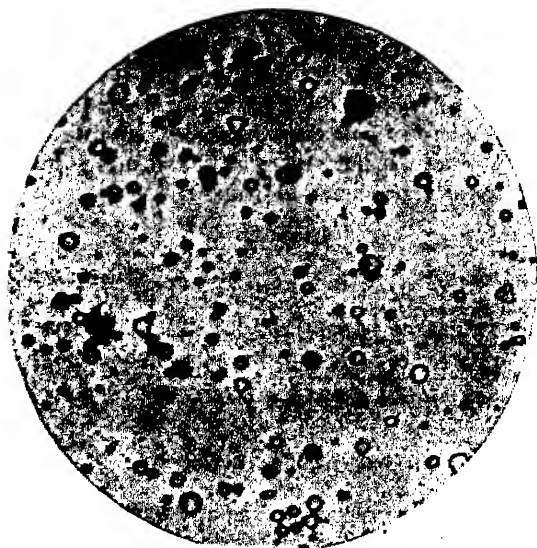
THEORY OF GRAIN GROWTH

These "breaks" can be recognized as approximately coincident with the appearance of coalescence of grains as observed in the accompanying photomicrographs. Figures 6a and 6b are from samples of grains obtained before the time of the "break" while Figures 6c and 6d are from samples removed from the ripening environment after the time of "break."

In explanation of this, the following tentative theory is proposed. In the first stage of digestion the process of Ostwald ripening is occurring, grains below a certain size are dissolving and grains above a certain size growing by accretion from solution. This process proceeds to exhaustion of the grains whose solubility is appreciably different from that of the largest. That is, the solubility difference $C - C_0$ has become negligible (Fig. 7). This would lead to practical cessation of grain growth, in a manner represented diagrammatically for a given dissolving class by Figure 8.

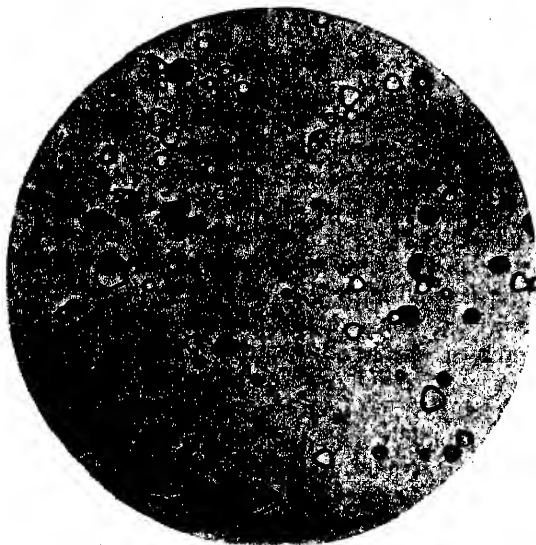
We find, however, that actually the grain growth goes on but at a new and decreased rate as illustrated in Figure 5. This we assume to be due to recrystallization within the aggregates produced by collisions and coalescence. It has been observed already that this coalescence appears to increase rapidly once the "break" in the growth curves has been reached. We suggest the following explanation of this.

In the pure Ostwald ripening each particle is surrounded by an electrostatic double layer. We regard this double layer as essentially a layer of imperfectly oriented ions of the solid plus ions of the solubilizing potassium bromide, these ions being more or less hydrated, according as they are less or more completely oriented in the crystal



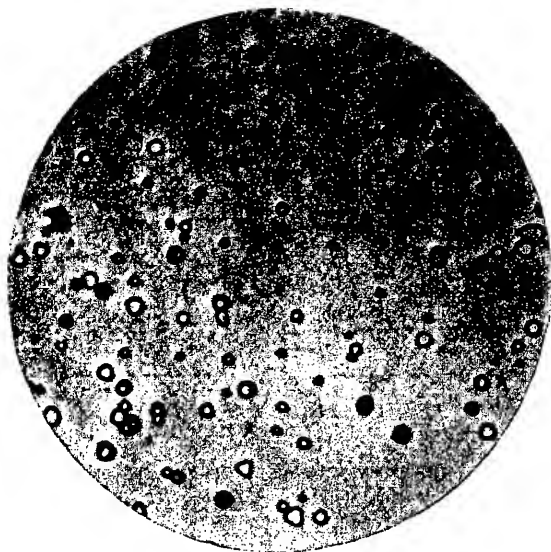
345 Min.

FIG. 6a.



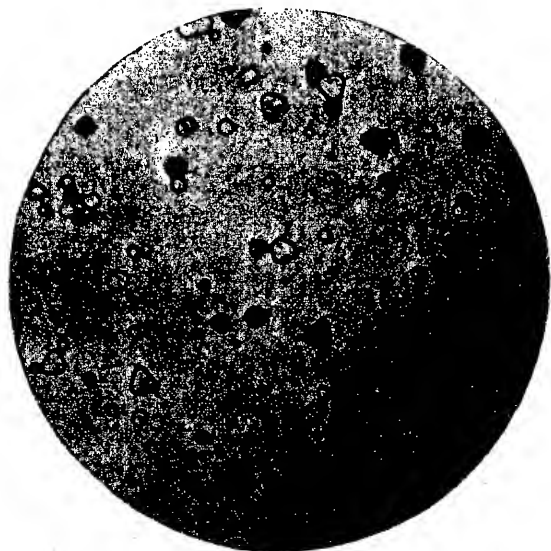
870 Min.

FIG. 6b.



495 Min.

FIG. 6c.



1110 Min.

FIG. 6d.

lattice. The hydration of each ion is supposed to consist of a monomolecular sheath of oriented water, H_2O , dipoles, which are more or less numerous, according as the potential electric moment of the ion is

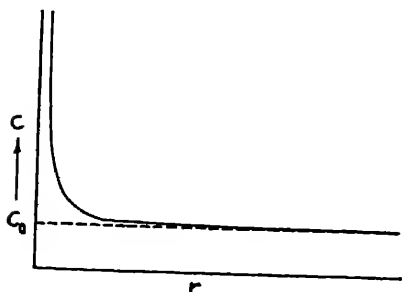


FIG. 7.

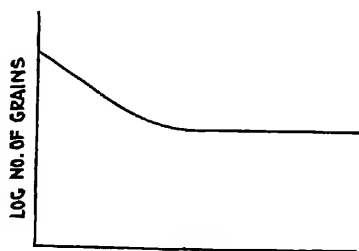


FIG. 8.

not taken up by its attraction and fixation in the crystal lattice. It is therefore a maximum at some distance in the solution from the crystal, and falls off very rapidly at the surface.

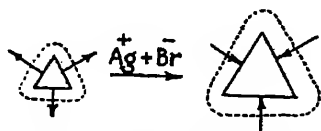


FIG. 9.—Ostwald Ripening.



FIG. 10.—Stage of No Growth.

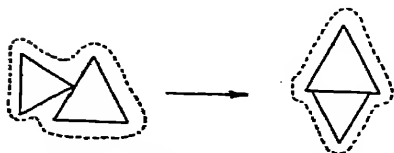


FIG. 11.—Coalescence and Crystal Orientation.

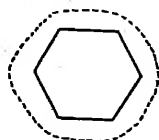


FIG. 12.—Recrystallization.

This seems compatible with both Gouy's⁹ theory of a gradually diminishing double layer—since presumably there is an influx and efflux of hydrated ions—and with McBains' theory of a monomolecular double layer, since the only dimension that can be attributed to it is that of the pairs of ions of opposite sign.¹⁰

In the stage of Ostwald ripening these layers are not in the same

⁹ Gouy, *J. Phys.* (4), 9, 457 (1910); *Compt. rend.*, 149, 654 (1909).

¹⁰ This may be considered for this case as a double layer of two pairs of oppositely charged ions since both $AgBr$ and KBr are in solution.

state for the dissolving particles as for the growing particles, since in the former the layer is being renewed from within, the latter from without (*cf.* Fig. 9). While the process of Ostwald ripening is proceeding, there is in principle a transfer of water from the growing grain to the dissolving grain, so that they are kept apart by the neutral water atmosphere thus created, so that very few collisions result in coalescence. When the process has declined, the double layers are similar in character as to hydration of the ions, and as to relative efflux and influx of ions out of and into the double layer (*cf.* Fig. 10). These can coalesce on collision like two similar liquid globules, *i.e.*, according to the fluctuation of the orientation and local surface charge giving a common double layer, and the two crystals—occasionally more—being in absolute contact (*cf.* Fig. 11).

The double layer then will tend to reduce its potential (interfacial) energy, thus bringing the crystals into a maximum possible contact (*cf.* Fig. 11). There is still further reduction of this potential energy possible, by recrystallization out of the double layer, *i.e.*, assuming migration of the imperfectly oriented silver and bromine ions of the double layer. This means a recrystallization, by which a single crystal tends to form from the aggregate crystal (*cf.* Fig. 12).

RELATION OF GRAIN GROWTH TO SIZE FREQUENCY

The general law for grain growth thus far discussed is of the form

$$-\frac{dn}{n} = k dt \quad [1]$$

where n is the number of individuals in a given size-class, and k is a factor affecting solubility. Thus in digestion in potassium bromide solutions, k becomes C^n , where C is the concentration of soluble bromide.

The implication of this with regard to the growth or change of class-size of an individual crystal is

$$\frac{dz}{z} = k_1 dt \quad [2]$$

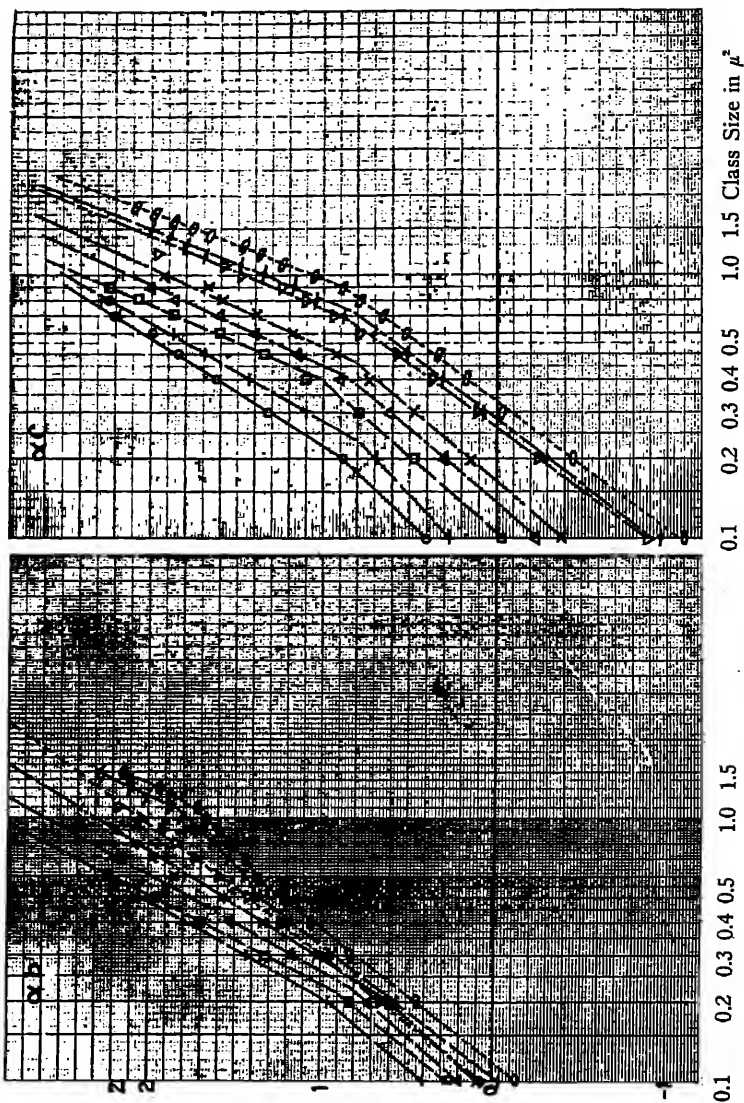
where z is the parameter of class-size to which n refers.

Since $f(r)$ is the parameter of size-frequency,¹¹ *i.e.*, of class-size, we should write this

$$\frac{df(r)}{f(r)} = k_1 dt \quad [3]$$

and $z = f(r)$ must be such, for the foregoing implication to hold, that we can substitute, *e.g.*, r^2 for r , without changing the fundamental

¹¹ We might speak of $f(r):n$ instead of size frequency.



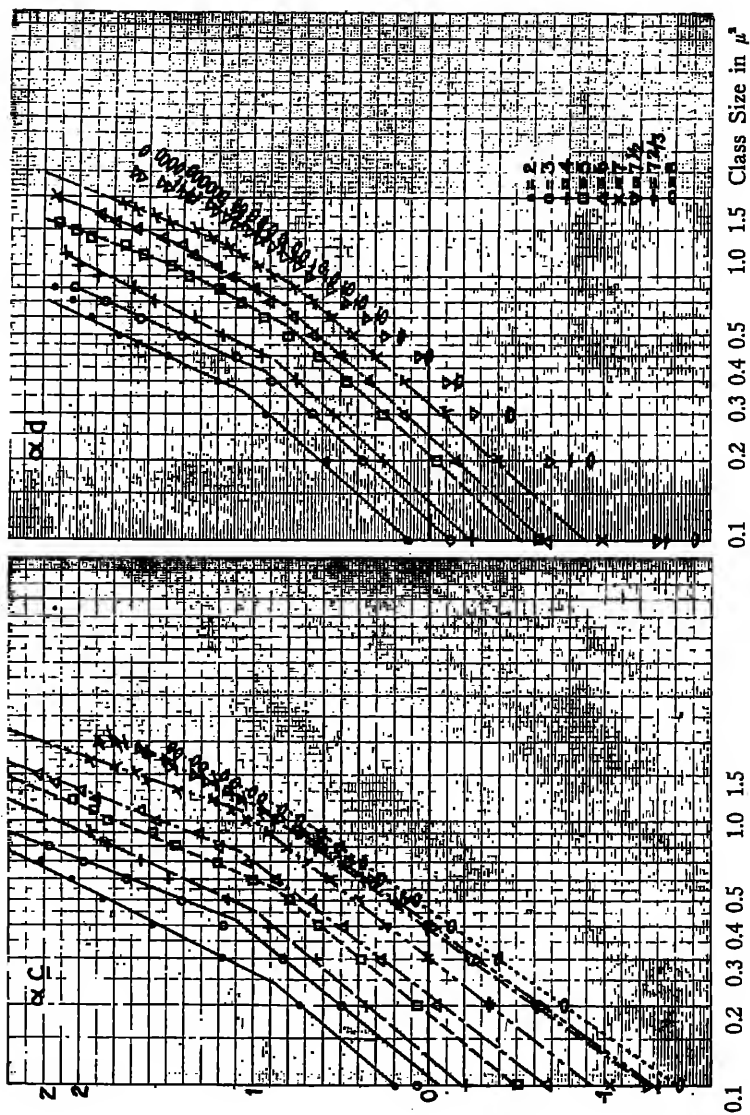


FIG. 13b.

form of the size-frequency law. A function which permits this is the Gaussian probability function with a logarithmic exponent as parameter

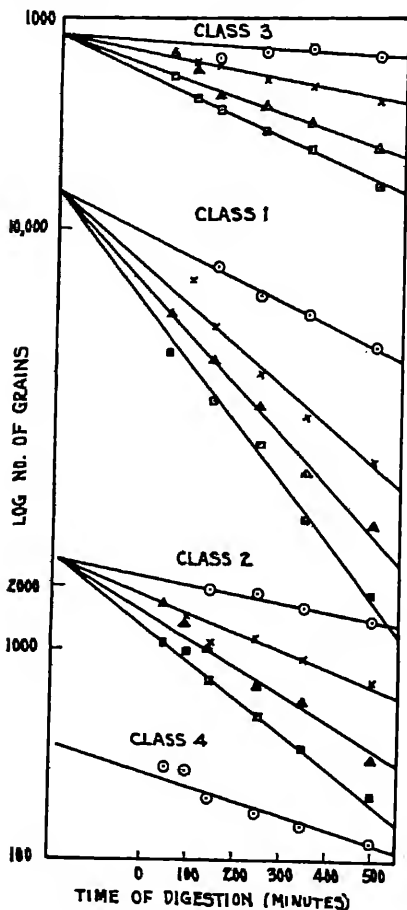


FIG. 14.

of the size.¹² We can substitute therefore $F(x) = F(r^2)$ in place of $f(r)$ and obtain the same size-frequency relations.

This function or frequency curve

$$y = Ac^{-k(\log x - a)^2}$$

[4]

¹² Kapteyn and Van Uyen, "Skew Frequency Curves," Hoitsema Bros., Groningen (1916); Wightman, Trivelli and Sheppard, *J. Phys. Chem.*, 28, 529 (1924).

has been found to represent the data for silver bromide precipitates over a considerable range, but with certain discontinuities or "breaks."¹⁸ These discontinuities are best brought out by graphs of the "normal" function. The normal function $z = \phi(x)$ is the summated frequency.

$$N \int_{x_1}^{x_2} \Omega(x) dx = y \quad (5)$$

When $y = \Omega(x)$ is actually given by equation 4, *i.e.*

$$y = \Omega(x) = Ac - k(\log x - a)^2$$

then plotting z on semi-log paper (abscissas $a \log x$), we should have a linear relation. (If z is plotted directly, we get a parabola.) When this is done for our data, results are obtained as shown in Figures 13a and 13b.

Each set ab , ac , ad and ae represents the different concentration of potassium bromide, and within the set the different times of digestion. These "normal" distribution functions are merely shifted parallel to themselves with time of digestion, and the discontinuities appear to be unaffected by time of digestion. They must have been present, therefore, in the original distribution previous to the digestion process, and probably consequent on something in the primary precipitation process itself, *i.e.*, the "nucleation" period.

The relation of the total distribution to the time of digestion is perhaps better brought out by plotting the size-frequency data on log-log paper, which gives a series of parabolas whose foci lie on a common straight line (*cf.* Fig. 3). If the straight line portions of the graphs of $\log n$ against time (*cf.* Fig. 14) before the "breaks" are protracted backward, the lines for each size-class at different concentrations of potassium bromide are found to meet at points which lie equidistant from the origin of time. This "negative time" value, of 180 minutes, is too large to signify any real induction period. It can have only a virtual significance, which may be related to the initial phase of nucleation, but this cannot be determined at present.

SUMMARY

To summarize, the most important conclusions from this investigation appear to be the following:

(a) The transition of the grain-growth process from Ostwald ripening to recrystallization of aggregate crystals following coalescence.

(b) The independence of the type of size-frequency distribution of bromide concentration (solubilizer) and time; probably of temperature also.

¹⁸ Loveland and Trivelli, *loc. cit.*

Addendum

Since completion of this paper an important article has come to hand by Sven Oden "On Precipitation" [*Arkiv Kemi. Min. Geo.*, 9, 38 pages (1926)] which discusses grain growth.

*Eastman Kodak Co.,
Rochester, N. Y.*

THE UNIFORM DISTRIBUTION OF CATALYSTS THROUGHOUT POROUS SOLIDS

By HARRY N. HOLMES AND ROBERT C. WILLIAMS

INTRODUCTION

The formation of precipitates throughout porous solids is usually far from uniform. Yet porous supports for catalysts useful in facilitating reactions in the gaseous phase are decidedly important.

Dipping such a porous solid into one of two reacting solutions and then into the other causes the resulting precipitate to cover only the outer surface of each particle, thus clogging the capillaries and interfering with diffusion into the interior. Patrick suggested the use of the reaction between solutions of sodium silicate and ferric chloride, for example. The gelatinous precipitate when properly dried and activated becomes a porous mixture of hydrated ferric oxide and hydrated silica, a useful catalyst.

Reyerson impregnated a dry silica gel with a solution of copper nitrate. After driving off the water he heated the product in contact with a stream of hydrogen. The copper oxide formed by heating was of course reduced to metallic copper by hydrogen. Reyerson¹ also secured a uniform coating (on silica gel) of platinum, palladium, silver and other metals less active than hydrogen, by reduction with adsorbed hydrogen. This gas was admitted to the thoroughly evacuated silica gel at a temperature of about -30° C. Apparently an appreciable amount of hydrogen was adsorbed, for, on admitting a solution of silver nitrate to the gel and allowing the temperature to rise, a coating of metallic silver was uniformly deposited throughout the gel.

THE SOLUBLE GAS PROCESS

We decided to secure uniformity of solid deposit in a porous support by first immersing the porous solid in a solution of the salt desired, drying, admitting a suitable water-soluble gas and finally immersing the porous solid containing the dry salt in water.

¹ Reyerson and Thomas, Colloid Symposium Monograph, Vol. 3, Chemical Catalog Co., Inc., New York, 1925, p. 99.

METALLIC SULFIDE DEPOSITS

Fragments of dry porous silica gel were soaked in a molar solution of silver nitrate (although any concentration may be used) and then dried and activated by heating for approximately one hour in a stream of dry air at 150° - 200° . After cooling, a stream of hydrogen sulfide was passed through the gel, filling the capillaries. Since all but a few per cent of water had been removed by activation, reaction between solid silver nitrate partly filling the capillaries and adsorbed silver nitrate was very slow.

Water was next added in sufficient amount to wet the solid thoroughly. Of course both silver nitrate and hydrogen sulfide dissolved and immediately reacted to form a precipitate of silver sulfide. Since the two reactants were distributed together into the innermost capillaries *before* reacting, the precipitated sulfide was formed throughout the porous gel. Other metallic sulfides such as those of lead and copper were readily formed in similar manner.

It is not feasible to adsorb hydrogen sulfide first and then immerse in the salt solution afterwards (somewhat similar to Reyerson's method with adsorbed hydrogen), for in that case precipitation occurs on the outer surface of the particles, clogging the capillaries and preventing adequate penetration of the salt solution into the inner capillaries. Another vital distinction is that hydrogen is practically insoluble. By the new process evacuation and extreme cooling are not needed.

The probable use of sulfide-coated gels is in preferential adsorption, rather than in catalysis.

METALLIC OXIDE DEPOSITS

The same principle applied in securing a sulfide coating was successfully applied in securing uniform deposits of metallic oxides. In other words, the porous solid was immersed in a solution of ferric chloride, for example, dried, activated, allowed to adsorb ammonia gas and then moistened with water.

The water finally added after diffusion of ammonia to the innermost capillaries converted this gas to ammonium hydroxide, dissolved the dry ferric chloride, and thus permitted precipitation of ferric hydroxide in the innermost capillaries.

Other metallic oxides, or their mixtures, may be thus deposited. Their use in catalysis of gas reactions when supported in silica gel, or other very porous solids, is obvious. These oxides may readily be reduced to the metals by reduction with hydrogen when heated sufficiently.

METALLIC DEPOSITS

The principle of delayed reaction can be used to secure uniformly distributed deposits of metallic platinum, palladium, silver, and other metals less active than hydrogen.

In depositing platinum on a silica-gel support, the gel was first immersed in, or moistened by, a slightly basic solution of Na_2PtCl_6 , then dried at 100° , cooled, and carefully moistened with formalin solution. Excess of this solution would wash out the platinum salt. Reduction by formaldehyde is slow at room temperatures, fortunately for the success of this method.

The two reagents are intimately mixed throughout the porous solid so that on raising the temperature to about 100° reduction (rapid at such a temperature) to metallic platinum takes place in the innermost capillaries.

Other reducing agents, such as sodium formate, hydrazine and tartaric acid have been successfully used instead of formaldehyde. The technique for a palladium deposit was essentially the same as above except that the palladous chloride did not need to be made basic. The high value of platinum and palladium in catalysis suggests great possibilities with the very porous silica gel as a catalyst support.

For silver the method used was as follows: The dry gel is immersed in a silver nitrate solution of desired concentration, dried not above 140° , cooled, and allowed to adsorb ammonia gas to form the silver-ammonia complex (necessary for aldehyde reduction). The gel was moistened with formalin solution and heated to 100° to effect rapid reduction. The coated gel was finally washed with hot water.

*Oberlin College,
Oberlin, Ohio.*

1. The first part of the document is a list of names and titles, including "The Hon. Mr. Justice" and "The Hon. Mr. Justice".

2.

3.

THE DEVELOPMENT OF THE ULTRACENTRIFUGE AND ITS FIELD OF RESEARCH

By J. B. NICHOLS

When Professor Svedberg came to the University of Wisconsin as visiting professor of colloid chemistry in 1923, he brought with him the idea of devising a centrifugal machine so constructed that its effect on a colloidal solution contained therein could be observed while the machine was in action. It was my good fortune to start the problem with him at Wisconsin and later to continue it with him at his home University, Upsala.

Just as at one time there existed the almost unexplored region from $1.1 \text{ m}\mu$ to $50 \text{ m}\mu$, between the soft x-rays and the far ultraviolet in the electromagnetic spectrum, there also existed the region $0.7 \text{ m}\mu$ to $10 \text{ m}\mu$ in the particle-radius "spectrum" where great uncertainty prevailed. The ultracentrifuge was developed in order to investigate this unexplored region between true solutions and colloidal dispersions.

The principle of the ultracentrifuge is illustrated in Figure 1. A transparent cell containing the solution to be studied is rotated at a speed sufficient to produce a strong centrifugal field of force and thus increase tremendously the effect of gravity on the solution. A beam of light passes up through the cell and renders the contents of the cell visible during the rotation, so that the variation in concentration along the length of the cell can be determined visually, or photographically in terms of photographic densities.

If the solution consists of equal-sized particles and the centrifuging is rapid enough the diffusion may be neglected; the particle or micelle size may then be determined by measuring the displacement of the boundary $x_2 - x_1$ in the time interval $t_2 - t_1$, using a modified form of Stokes' law. With non-uniform material, however, a partial separation is effected giving us an opportunity to determine the distribution of sizes present, from analysis of the concentration-distance curve obtained.¹

With very small particles or large molecules it is rarely possible to neglect diffusion. When pure solvent is obtained between the meniscus

¹ Svedberg-Nichols, *J. Am. Chem. Soc.*, 45, 2910 (1923); Svedberg and Rinde, *ibid.*, 46, 2677 (1924).

and the boundary of the disperse phase in a time short compared with the total time, then a simple diffusion correction may be applied.² If a solution of even relatively large particles is subjected to prolonged centrifuging an equilibrium is finally established between the centrifuging and the counter diffusion of the particles from the outer end of the tube, similar to the Perrin sedimentation equilibrium in a gravity field.

Depending on the experimental conditions, micellar weights or size of particles may be determined either by the establishment of a sedimentation equilibrium between centrifuging and diffusion, or by the measurement of the sedimentation velocity.³

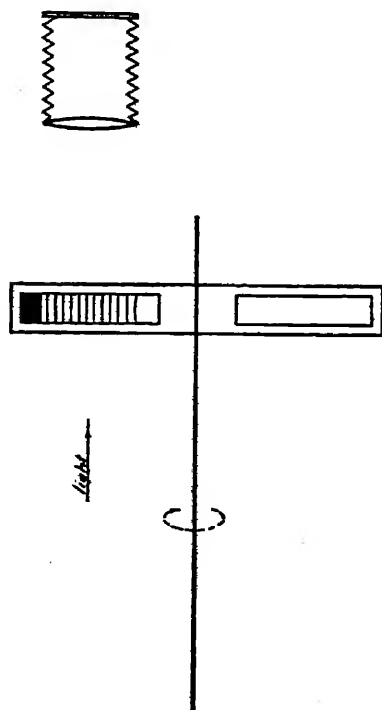


FIG. 1.

A centrifuge developing a centrifugal force of from 1000-10,000 times that of gravity would enable us to study the sedimentation velocities of the lyophobic inorganic colloids and the sedimentation equilibria of the proteins and other lyophilic substances of molecular weight greater than 10,000. For the determination of the sedimentation velocities of lyophilic substances of molecular weight greater than 15,000 and for the sedimentation equilibria of substances in the molecular range 10,000-1,000, centrifugal forces of about 100,000 times that of gravity are required.

The most important conditions that the ultracentrifuge must fulfill to give reliable data are: First, no vibrational or thermal effects should disturb the sedimenting system; second, the amplitude of vibrations of the rotor must be small enough and the optical system well enough

defined so that sharp pictures can be obtained; third, the substance must undergo no deterioration during the centrifuging; and fourth, really a corollary of the third condition, the provision should be made of low-temperature facilities for unstable materials.

² Svedberg and Rinde, *ibid.*, 46, 2682 (1924).

³ Svedberg, *Kolloid-Z. (Ergänzungsband)*, 36, 57 (1925).

Since the conditions for efficient operation were not well understood at the commencement of the work, the first machine devised was necessarily crude and did not satisfactorily produce centrifugal fields greater than 200 times gravity. In fact, its chief value lay in the orientation it gave to the problem. Freedom from vibration rather than temperature uniformity was thought to be of first importance for correct sedimentation conditions; therefore insufficient provision was made for the maintenance of temperature equality throughout the sedimenting system. Furthermore, long columns of solution were used—about three inch lengths—which would have been very difficult to keep at uniform temperature even if temperature regulation had been employed. In addition, the parallactic errors were appreciable in so long a column.

To test the action of the centrifuge a series of fairly uniform gold sols and clay sols, of ultramicroscopically determined size of particle, were studied.⁴ The results obtained, while they must have been in part vitiated by convection currents,—the double boundaries obtained for barium sulfate and arsenious sulfide were undoubtedly due to this disturbance—proved that under proper conditions the method should be applicable to the study of fine-grained colloids.

With the problem thus oriented, Svedberg on his return to Sweden undertook a thorough study of the conditions necessary to the proper functioning of the centrifuge. Such important advances in apparatus and in technique were made that Rinde⁵ was able to determine distribution curves for even the most highly dispersed gold sols, the Faraday sols, which give only a very faint Tyndall cone in the ultramicroscope. Moreover, preliminary experiments indicated that even proteins could be centrifuged; in other words, the method was already sufficiently refined so that extremely valuable information could be obtained as to their molecular nature and uniformity.

The ultracentrifuge as originally designed by Svedberg and Rinde was of the self-balancing type, the top of the shaft having a certain curvature so that the rotor could assume a position of true balance with the minimum of effort as the centrifuge was attaining its final speed. Uniformity of temperature throughout the liquid proved to be of prime importance. A very slight temperature difference was sufficient to set up injurious convection currents. Two sources of disturbing heat effects were found to lie in the heat generated by (1) the rotor acting against the surrounding air and (2) the friction of the bearings. When the rotor was run in an atmosphere of hydrogen and the temperature of the bearing nearest the rotor was regulated by circulating water through it, the convection currents were much diminished, but still persisted, espe-

⁴ Svedberg and Nichols, Ref. 1.

⁵ Svedberg and Rinde, Ref. 1.

cially if the dissolved substance was not greatly different in density from the solvent. A slight evaporation from the surface of the solution into the air space of the cell was found to be the agent; therefore, when the meniscus was covered with a thin layer of vacuum oil, temperature uniformity could be maintained for days if necessary.

The characteristics of the centrifuge cells were also much improved. The final type of cell is made of three circular glass or quartz plates cemented together. The two outer plates form the windows and the middle plate has a sector aperture of a 5° angle cut out of it to make the proper shape of cell to give uniform exposure outward along its length as the peripheral velocity increases. These cells are cemented in steel shells and fastened in place in wells provided for them in the rotor.

The photographic recording of the effect of centrifuging depends on the substance possessing an absorption band in the spectral region of the illuminant. Since many substances are colorless the technique was next developed⁶ for studying them in the short ultra-violet region of the spectrum where most substances have an absorption band. A quartz optical system was used together with gaseous chlorine and bromine filter cells to isolate the 254 $m\mu$ mercury line for illumination. To adjust the light intensity to the same value for a series of exposures a standard solution was photographed at the same time as the substance under study.

With these improvements relatively stable lyophilic substances of as small micellar weight as the proteins could be studied by means of the sedimentation equilibrium but the sedimenting system could not be thermostated at any desired temperature (for example at 0° C. for unstable proteins) and the maximum centrifugal force developed, 7000 times gravity, was not high enough to give very accurate information when mixtures of micelles of not very different weight were present in a solution.

Making use of the best mechanical features of the previous type, a new ball-bearing thermostated centrifuge has recently been developed; an improved Baltic cream separator is used as the basis since it is so readily made water tight. Figure 2 shows a view of this centrifuge from the top, with the lid of the rotor chamber removed. The rotor chamber is surrounded by a lagged water bath, the temperature of which can be regulated as desired. For 0° C. the outside surfaces of the windows of the rotor chamber are electrically heated to prevent the condensation of moisture and the consequent impairment of their optical properties. Thus very unstable substances that would decompose or change at ordinary temperatures can now be investigated.

The sedimentation equilibrium method is sounder thermodynamically

⁶ Svedberg and Nichols, *J. Am. Chem. Soc.*, 48, 3081 (1926).

cally, but requires on the average two days to reach a steady state for an 0.5 cm. length of column. On the other hand, the sedimentation-velocity method, if sufficiently high speed is available, gives information in two or three hours for substances of micellar weight of the order of 50,000. It is also possible to obtain more exact information when a mixture of micelles is present in a solution because a greater transient separation occurs in the unsteady state.

The most important conditions that this "molecular" centrifuge should fulfill are: A centrifugal force in the cells 80,000-100,000 times that of gravity; cells well enough constructed to stand 100-200 atmospheres pressure; temperature difference between the rotor and the casing

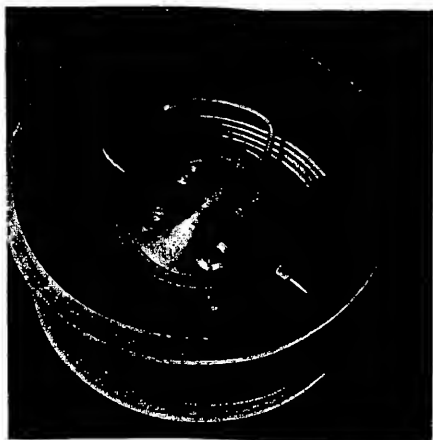


FIG. 2:

as small as possible to avoid the production of convection currents; possibility of regulating the temperature of the sedimenting system; amplitude of vibrations small enough so that sharp photographs could be taken during centrifuging at high speed; optical parts inside the centrifuge remaining free from oil dust.

Svedberg and Lysholm,⁷ after a two years' study of the possibilities, developed the oil turbine type of ultracentrifuge to meet the above conditions. Figure 3 shows the rotor and Figure 4 gives a diagrammatic representation of the centrifuge. The rotor is made from a solid block of chrome nickel steel and has openings for four cells. It is driven by high-pressure oil impinging against two eight-bladed oil turbines, one at each end of the shaft. The rotor revolves in hydrogen gas at a reduced pressure (15 mm. was found to be the optimum; whereas the

⁷ *Nova Acta Reg. Soc. Scient. Upsalienis*, Vol. ex. ord., ed. (1927).

work done on the gas decreases continuously with reduction of pressure, the heat conductivity of hydrogen is constant down to 20 mm. Hg but thereafter falls off rapidly). At this optimum pressure there was only 1.5° temperature difference between the rotor and the casing. The oil from the bearings is prevented from entering the rotor chamber by an elaborate system of oil rings and deflectors. Bearing and casing temperatures are measured by thermocouples and the temperature is adjusted by cooling the oil before it is admitted to the turbine chambers. An observation channel is bored through the end brackets and top casing and closed by round glass or quartz windows protected from oil dust by electromagnetically controlled shutters. In order to eliminate any



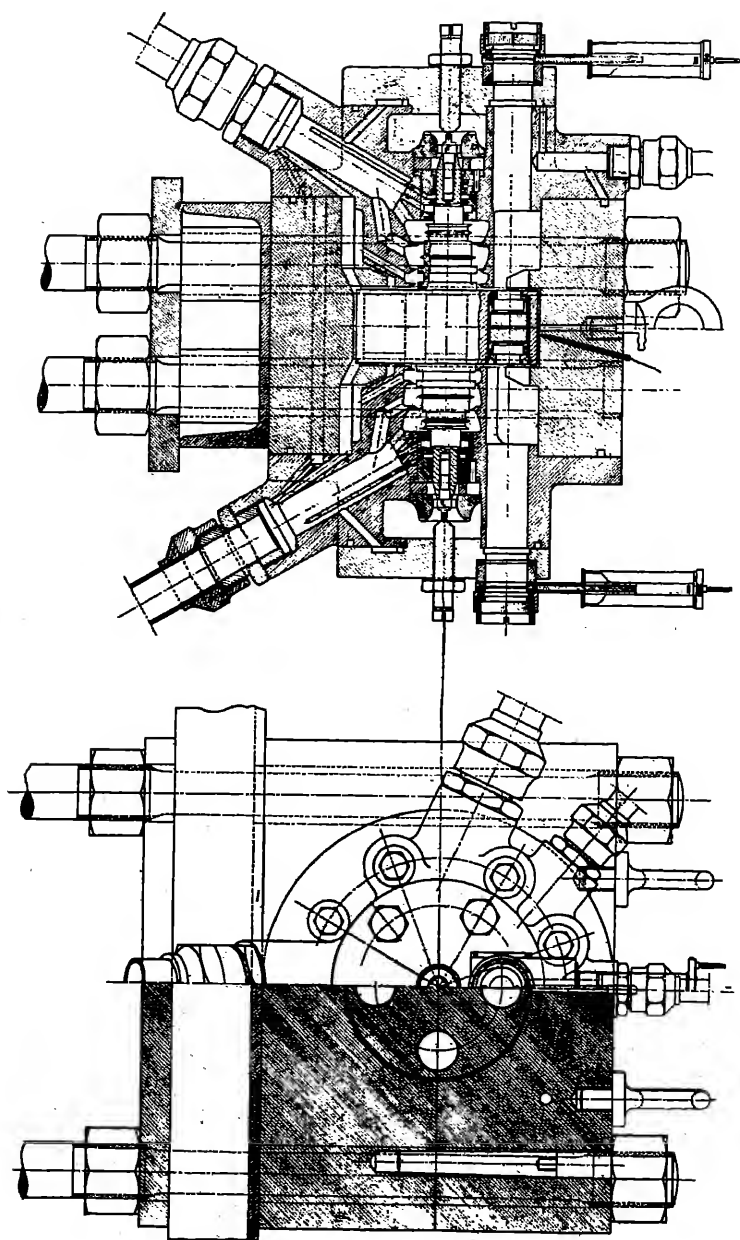
FIG. 3.

gearing arrangement the speed of the rotor is determined by means of a stroboscopic tachometer.

The complete oil and hydrogen circulation system is shown in Figure 5 and is self-explanatory. The oil is pumped from the large oil container *C* through the oil cooler *D* to the centrifuge and then back to the oil container. Part of the oil, however, lubricates the bearings and drains down into the lower container *H* from which it is pumped through the filter *K* to the main container.

In the foregoing pages the development of the ultra-centrifuge from its inception to a research tool of increasing precision has been sketched and the conditions necessary for successful centrifuging discussed. The following pages will give some indication of its wide field of usefulness.

FIG. 4.



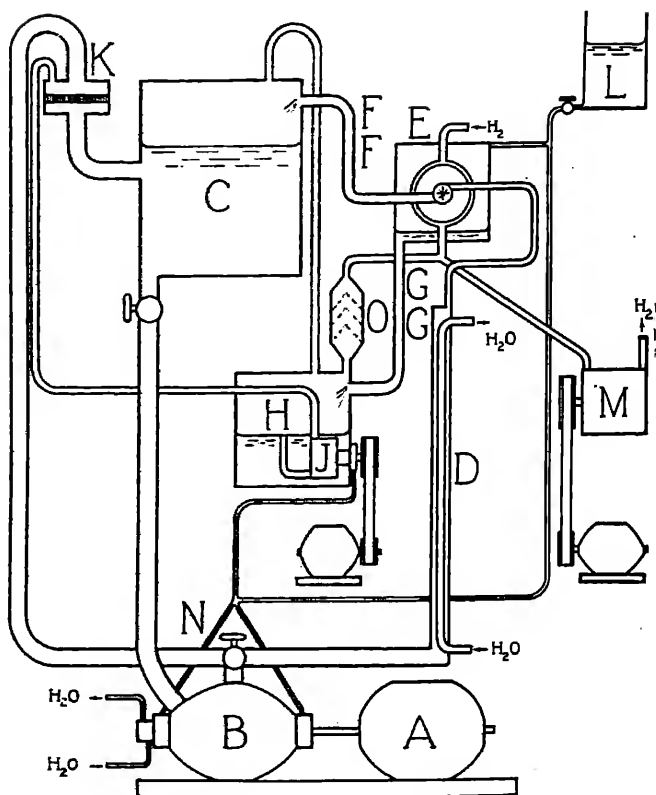


FIG. 5.

THE FIELD OF RESEARCH

Let us now consider the possibilities of the two methods of investigation which the ultracentrifuge offers. For complex molecules the sedimentation equilibrium is expressed by the relation ⁸

$$M = \frac{2RT \ln \left(\frac{c_2}{c_1} \right)}{(1 - V\rho) \omega^2 (x_2^2 - x_1^2)} \quad [1]$$

where M is the molecular weight, R the gas constant, T the absolute temperature, V the partial specific volume of the substance, ρ the density of the solution, ω the angular velocity and c_2 and c_1 the con-

⁸ Svedberg and Fahraeus, *J. Am. Chem. Soc.*, 48, 430 (1926); Svedberg and Nichols, *Ref. 6*.

centrations at the points x_2 and x_1 distant from the axis of rotation of the centrifuge. This is a true thermodynamic method, depending as it does on the establishment of an equilibrium between centrifuging and diffusion of the substance. On the other hand, the sedimentation velocity method may be styled "kinetic" because the displacement of the particles in a given time interval is measured.⁹ For small x -intervals:

$$M = \frac{RTs}{(1 - V\rho)D} \quad [2]$$

or for large intervals

$$M = \frac{RT \ln \left(\frac{x_2}{x_1} \right)}{(1 - V\rho)D\omega^2(t_2 - t_1)} \quad [2a]$$

Here $s = \frac{1}{\omega^2 x} \cdot \frac{dx}{dt}$ is the specific sedimentation velocity, a characteristic constant for each molecular species or particle size for a given temperature and solvent, D is the diffusion constant of the substance.

Inspection of equations [1] and [2] shows that no assumptions have been made as to form and structure of the molecule. The molecular or micellar weight can therefore be determined with the same freedom from hypothetical quantities as in the classical methods.

The ordinary methods of molecular weight determination cannot be applied with certainty for values greater than 1000, not only on account of their insensitiveness but also because small amounts of impurities of low molecular weight introduce such large errors. Osmotic pressure measurements have been used to estimate molecular weights of complex substances but owing to the many inherent sources of error, such as electrokinetic phenomena at the membrane, permeability to the solute, Donnan equilibria and the resulting membrane potential, membrane hydrolysis and the like, the method has been in disfavor until the recent careful measurements of Adair¹⁰ and others on hemoglobin. The ultracentrifuge is free from membrane errors and, more important, produces a partial separation of different molecular species, thus enabling us to determine their relative concentrations.

Diffusion measurements have also been used to evaluate molecular weights of proteins and other complex substances. It is necessary to assume the validity of the Einstein diffusion equation or a semi-empirical relation to carry out this evaluation; however, since many of the complex molecules are not isotropic such a calculation can ordinarily give only the order of magnitude of the molecular or micellar weight. If the

⁹ Svedberg, *Z. physik. Chem.*, 127, 51 (1927); Svedberg and Nichols, *J. Am. Chem. Soc.*, 49, 2920 (1927).

¹⁰ *Proc. Roy. Soc.*, 108A, 627 (1925); 109A, 292 (1925).

molecule or particle is solvated or holds other adsorbed material the estimation may be far from correct.

Hemoglobin is not an isotropic molecule but may be more or less plate-shaped. Its normal diffusion constant, as determined by centrifuging,¹¹ is 0.071 cm.² per day at 30° C., whereas its diffusion constant from the Einstein equation on the basis of the molecular weight of hemoglobin, 68,000, should be about 0.084 cm.² per day at 30° C. The value of 0.071 cm.² per day used to calculate the molecular weight from ordinary diffusion measurements would give 41,000. The value 0.0645 for hydrated carbon monoxide-hemoglobin at a pH of 9.05, if substituted in the Einstein equation, would give a molecular weight of 30,700, whereas actually the molecular weight is still 68,000 at this alkalinity.

Tendeloo¹² has advanced Smoluchowski's electro-viscosity equation as a means for estimating the size of colloidal particles or micelles. However, the use of this equation requires a knowledge of several quantities difficult to determine experimentally, such as the electrokinetic potential, so it is not probable that more than an orientation can be obtained by this method.

X-ray methods occupy a prominent place at present but as yet give only approximate information concerning the size of nitrocellulose micelles or complex molecules like egg albumin. They indicate a periodicity, it is true, but usually the unit cell is^a far smaller than the complete molecule or micelle. Katz finds a relatively small unit cell for hemoglobin, approximately $a = 5.6\text{\AA}$, $b = 30\text{\AA}$, whereas the ultracentrifuge shows it has a molecular weight of 68,500, corresponding to an equivalent sphere 2.7 μ in radius. Similarly the unit cell of cellulose is $10.2 \times 8.45 \times 7.9\text{\AA}$, while the crystallite may be more than 1000 times as large according to Herzog's data¹³ from the broadening of the diffraction rings and diffusion measurements. The position of the lines of the x-ray diagram remains the same during the process of depolymerization or degradation of the cellulose crystallite until the configuration of the unit cell is altered, but it is relatively easy to follow the whole process with the ultracentrifuge until unit cell dimensions are approached. Thus the data given by the ultracentrifuge serves to complement the results obtained by the x-ray method.

Aside from the general function of the ultracentrifuge to determine molecular weights or particle size and the distribution curves by means of the partial separation it produces, what can we accomplish with the apparatus? First, the ultracentrifuge furnishes practically the only method of studying some of the thermodynamic properties of complex substances. Second, with a sufficiently high centrifugal field of force

¹¹ Svedberg and Nichols, *J. Am. Chem. Soc.*, 49, 2932 (1927).

¹² Tendeloo, *Kolloid-Z.*, 41, 290 (1927); *Chem. Weekblad*, 25, 158 (1928).

¹³ *J. Phys. Chem.*, 30, 457 (1926).

available, the velocity of sedimentation and the diffusion constant may be determined separately thus leading to the detection of certain anomalies that occur in these quantities when the substance is subjected to different environments.

THERMODYNAMIC APPLICATIONS

The most important thermodynamical properties which may be obtained from the sedimentation equilibrium are (1) the equilibrium constant for a chemical equilibrium between large molecules and the effect of centrifugal force, pressure and temperature on the equilibrium, (2) the electrical potential resulting from the partial separation of colloidal ions by centrifuging, and (3) the determination of the activity of solute and solvent in concentrated solutions.

If the sedimentation equilibrium attained by a complex substance, a dyestuff or a protein, for example, indicates that more than one molecular species is present, it is possible to decide whether a simple mixture of molecules obtains or whether there is a chemical equilibrium. The latter state is distinguished from a simple mixture by centrifuging different concentrations of the sample. If the same distribution is obtained for each concentration, then a mixture is present; but if the distribution obtained varies with the original concentration of the material centrifuged then there is an aggregation equilibrium.

When the molecules are in equilibrium, the equilibrium constant can be determined and, of course, from this quantity the free energy change involved in the reaction. Furthermore, the effect of pressure, temperature and centrifugal force on the equilibrium constant can be studied. Tiselius¹⁴ has treated theoretically the question of a reaction occurring in a system under the influence of a centrifugal potential and finds that if there is no change in the specific volumes as a result of the reaction then the equilibrium constant is independent of the centrifugal force and can be calculated for the pressure p' existing at any point x in the centrifuge from the concentrations found for the two substances from the analysis of the sedimentation equilibrium. It should be mentioned in passing that the pressure p' at a given point in the centrifuge is not the ordinary pressure but increases with the centrifugal force and the distance from the meniscus of the solution. Thus, if a fairly compressible substance is reacting, which causes the equilibrium constant to vary appreciably with pressure, the variation of the equilibrium constant may be determined from one centrifuging by calculating its value for different distances from the axis of rotation. In case there is only a small variation with pressure, then centrifugings at sufficiently different speeds to give widely different pressures will give the desired data.

¹⁴ *Z. physik. Chem.*, 124, 454 (1926).

If there is an appreciable volume change in the reaction then the equilibrium constant will vary with the centrifugal force, as Tiselius has shown. The equilibrium constant must be multiplied by an exponential factor containing the centrifugal force and the change in specific volume on account of the reaction. For ordinary substances this change in volume is too small to be measured, but for many complex substances the change may be relatively large.

When charged particles or ionized molecules are present in a solution, centrifuging produces a partial separation of charges, thus introducing an electrical potential related to the Donnan membrane potential acting in opposition to the centrifugal potential.¹⁵ Here, however, the electrical forces are more easily treated because there is no semi-permeable membrane as in the osmotic method to introduce anomalies. For the simplest case of a solution of a completely dissociated colloidal electrolyte containing no other electrolytes, both the sedimentation equilibrium and the sedimentation velocity relation will correspond to a molecular weight $(n + 1)$ times as small as the true molecular weight,¹⁶ where n is the charge or valence. This is the maximum effect, however, and is of slight importance because it is almost never realized. There will generally be a small amount of electrolyte present as impurity, or, when the electrical potential arises, a slight hydrolysis may occur. A trace of electrolytic impurity represses this limiting potential exceedingly so that the potential rarely amounts to more than a few millivolts between the top and the bottom of the solution under the most favorable conditions. If sufficient electrolyte is added the Donnan potential can be almost totally repressed. Thus by the use of suitably concentrated buffer solutions it is possible to submit the whole sedimenting system to a definite acidity and salt concentration.

Since we are able to subject the whole system to uniform conditions, it is possible to detect deviations from the gas laws. The substance must first be studied in sufficiently dilute solution to determine the molecular weight or particle size where the gas laws are valid; then we can proceed to more concentrated solutions and obtain very valuable data on the activities of the substances. The factors influencing the activity can be ascertained as well. Having obtained the activity of the solute, it is easy to calculate the activity of the solvent, either in the ordinary way¹⁷ or by a special graphical method.¹⁸

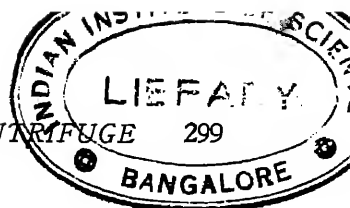
With the activity of the solvent determined, it is possible to calculate the osmotic pressure between two solutions even though the gas laws are not valid.

¹⁵ Tiselius, Ref. 14, p. 457.

¹⁶ Svedberg, *Kolloid-Z.*, (Ergänzungsband), 36, 63, 64 (1925).

¹⁷ Lewis and Randall, "Thermodynamics and the Free Energy of Chemical Substances," McGraw-Hill Book Co., New York, 1923, p. 268.

¹⁸ Tiselius, *Z. physik. Chem.*, 124, 459 (1926).



THE DEVELOPMENT OF THE ULTRACENTRIFUGE 299

KINETIC APPLICATIONS

Let us turn now to the "kinetic" quantities which can be determined by subjecting the solution to a sufficiently high centrifugal potential so that diffusion is rendered less important and the sedimentation velocity and the diffusion constant may be determined separately. In this manner, effects may be detected that react both on the rate of diffusion and the rate of sedimentation; effects that refer more directly to the viscous forces acting on the particles would cancel out or be disguised in the sedimentation equilibrium.

For the sake of clearness, the various possibilities are brought together in Table I, assuming that the normal values of the diffusion constant D and the specific sedimentation velocity s have been determined in sufficiently dilute solution for the gas laws to hold.

TABLE I.¹⁹

D	s	Effect
Reduced in the same ratio		Hydration or solvation
Reduced.	Between hydration reduction and normal value.	Adsorption of a substance of density such that $\left(\frac{\rho_c - \rho}{\rho_p - \rho}\right) > \left(\frac{r}{R_1}\right)^2$
Reduced.	Increased.	Adsorption of a substance of density such that $\left(\frac{\rho_c - \rho}{\rho_p - \rho}\right) < \left(\frac{r}{R_1}\right)^2$
Same effects as for adsorption, but in general of lower magnitude for particles, much lower for molecules.		Compound formation.
Much increased and increases with time.	Decreased but may increase with time.	Splitting of the molecule or particle.
Much increased and probably constant.	Decreased and probably constant.	Donnan effect.
Pure Donnan effect D and s values decreased in the same ratio, as a first approximation.		Hydration and Donnan effect.
Much reduced but approaches normal value as concentration is decreased.	Slightly reduced but becomes normal at lower concentrations.	First stages of gel formation or van der Waals effects.

When hydration occurs, if the solvent molecules attached to the particles retain their normal density, then both the diffusion constant

¹⁹ Cf. J. B. Nichols, Doctor's Thesis, University of Wisconsin, 1927, p. 25 *et seq.*, for a fuller treatment of the factors brought together in this table.

and the sedimentation velocity are reduced by the same factor $\frac{r}{R_1}$, where r is the radius of the unsolvated particle and R_1 is the radius of the solvated particle.

In the case of adsorption, the diffusion constant is reduced by the same factor $\frac{r}{R_1}$ but the sedimentation velocity is affected by the factor $\frac{\rho_c - \rho}{\rho_p - \rho}$ where ρ_c is the density of the adsorption complex, ρ_p the density of the original particle and ρ the density of the solvent. Thus

$$s_{obs} = \left(\frac{R_1}{r}\right)^2 \cdot \left(\frac{\rho_c - \rho}{\rho_p - \rho}\right) s$$

Qualitatively, the Donnan effect acts in the same way as the splitting up of the particles or molecules, that is, a large increase in the observed diffusion constant and a smaller decrease in the observed specific sedimentation velocity. The exact theory has not as yet lent itself to treatment but it seems probable from experimental results that these quantities are constant for the Donnan effect. On the other hand, an increase with time in the value obtained for the diffusion constant should indicate the presence of more than one molecular species. Thus, for an equimolecular mixture of weights 34,000 and 68,000, the observed D should be 0.0964 after two hours of centrifuging at a speed of 39,000 r.p.m., while after three hours it should be 0.1073. However, in case a mixture is present which causes only a slight increase in D with time, the drift may be masked by experimental errors.

APPLICATIONS TO PROTEINS

To illustrate some of the points brought out in the previous discussion, the investigation on the proteins carried out in Svedberg's laboratory during the past three years will serve as an example.

Until recently so little was known concerning the effect of salts and hydrogen-ion concentration on the molecular condition of hemoglobin that various theories had arisen to explain the form of the oxygen dissociation curve. Ultracentrifuge studies by Svedberg and Fåhræus²⁰ show conclusively that carbon monoxide-hemoglobin is a uniform substance of molecular weight 68,000 in electrolyte-free solution, that salt solution does not cause aggregation, and that oxy- and met-hemoglobin have the same molecular weight as the more stable carbon monoxide form. In addition, Svedberg and Nichols²¹ have demonstrated that at

²⁰ *J. Am. Chem. Soc.*, 48, 430 (1926).

²¹ *Ibid.*, 49, 2920 (1927).

least over the pH range of 6.0 to 9.05 carbon monoxide-hemoglobin exists as a single molecular species. Figure 6 gives the variation of concentration with distance after 0.5, 1.0, . . . 3.0 hours of centrifuging of a 1 per cent carbon monoxide-hemoglobin solution buffered at a pH of 7.56 with primary-secondary phosphate buffer. The solution was subjected to a speed of 39,000 r.p.m. at 30° C., corresponding to a centrifugal force 85,000 times that of gravity. The dotted curves for 2.0 and 3.0 hours represent the theoretical diffusion curves of a uniform

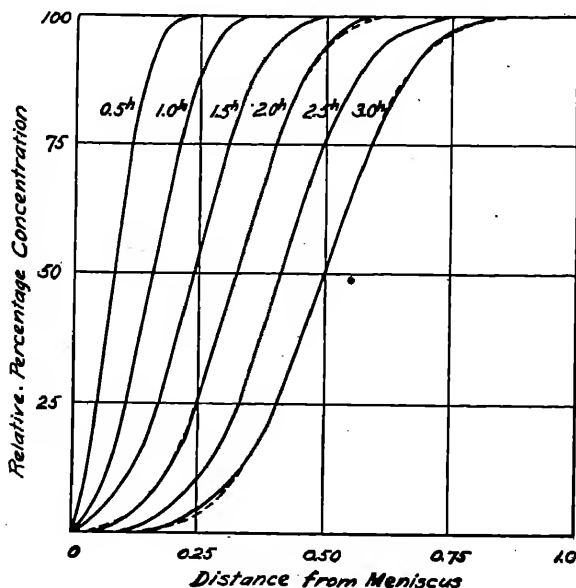


FIG. 6.—Centrifuging of Hemoglobin.

substance of molecular weight 68,000, if subjected to the same experimental conditions. The agreement leaves little room for doubt that hemoglobin is a unimolecular substance.

Therefore, Haldane's assumption²² that reduced hemoglobin is more aggregated than oxyhemoglobin and Barcroft and Hill's postulate²³ of a unimolecular form of hemoglobin in distilled water and a polymeric form in salt solutions prove to be unfounded. Henderson's suggestion²⁴

²² Haldane, "Respiration," Yale University Press, New Haven, 1922, p. 80.

²³ Hill, *J. Physiol.*, 40, p. IV (1910); Barcroft, "The Respiratory Function of the Blood," Cambridge, 1914, p. 59; Barcroft and others, *J. Physiol.*, 56, 157 (1922); Brown and Hill, *Proc. Roy. Soc.*, 94B, 257 (1923).

²⁴ *J. Biol. Chem.*, 41, 401 (1920).

is therefore rendered more probable that the S-shaped oxygen dissociation curve may be due to changes in base bound on oxygenation.

Hemoglobin has absorption bands in the yellow, green, blue, long ultra-violet and short ultra-violet regions of the spectrum. A thoroughly purified sample gives the same molecular weight for all of these bands but an undialyzed sample gives a different value in the short ultra-violet than in the visible regions, due probably to the presence of some globulin. This fact immediately suggests the possibility of determining the conditions that exist in a mixture of several substances having different absorption bands. By illuminating the sedimenting system with light corresponding to the different absorption bands each substance could be determined separately. In this manner, hemoglobin and serum albumin could be studied to ascertain if there was any interaction. Or, with a knowledge of the individual molecular weights of serum albumin and serum globulin, diluted serum could be studied directly, with less chance of change taking place than in the long purification process, to see if the mixture of proteins showed indications of combination or equilibria in the serum.

One great advantage of the centrifugal method lies in the fact that by choosing a suitably strong absorption band, accurate results can be obtained with difficultly soluble substances. Even if there is no strong band in the ordinary region of the spectrum, almost all substances absorb strongly below $\lambda = 250 \text{ m}\mu$, so the 254 $\text{m}\mu$ line of the mercury arc or the 220 $\text{m}\mu$ line of a cadmium spark is suitable for illumination.

The molecular weights of phycoerythrin and phycocyan from sea weed *Ceramium rubrum*, studied by Svedberg and Lewis,²⁵ could have been determined in no other way because these proteins are difficultly soluble and have no metallic constituent. Similarly, Svedberg and Chirnoagă²⁶ were able to extend their measurements on hemocyanin from *Helix pomatia* to sufficiently dilute solution, 0.05 per cent, so that conditions became normal and they could ascertain that there is but one molecular species present of molecular weight 5,000,000. Thus the molecule apparently is large enough to function as the corpuscle in the blood stream of the snail. Perhaps such huge molecules should more properly be called "bio-molecules" to indicate that in spite of their uniformity they possess functional characteristics.

Figure 7 shows the variations that the diffusion constant and the sedimentation velocity of hemocyanin undergo with concentration. With such huge molecules, at higher concentrations intermolecular forces probably tending to gel formation enter, thus preventing a completely free diffusional movement and decreasing the diffusion constant. On the

²⁵ J. Am. Chem. Soc., 50, 525 (1928).

²⁶ Ibid., p. 1399.

other hand, since there is no actual combination the sedimentation velocity should not change much. It should be noted that over this concentration range normal conditions prevail for egg albumin and hemoglobin but unlike hemocyanin neither of these substances forms a gel.

It is interesting to compare the appearance of sedimenting solutions of the most complex and of the simplest protein thus far studied. Figure 8 is a photographic reproduction of a 3.05 per cent solution of hemocyanin at a pH of 8.0 centrifuging in a field of force 5,900 times that

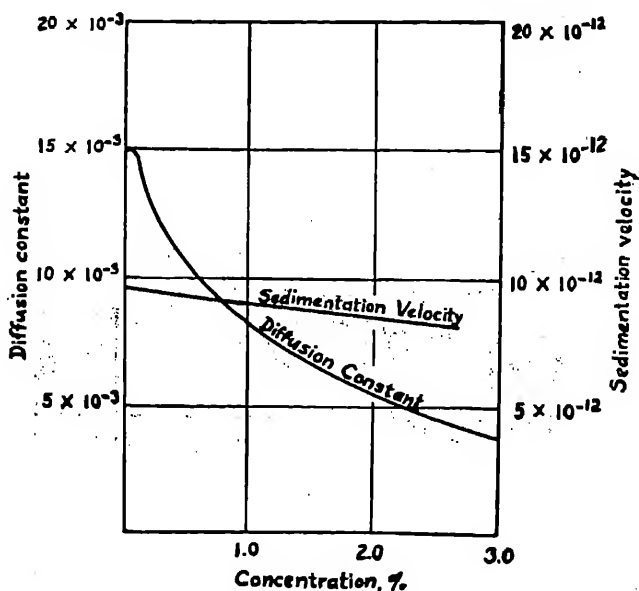


FIG. 7.—Centrifuging of Hemocyanin (Chirnoagă).

of gravity (11,000 r.p.m.) at a temperature of 18.5° C. The exposures were taken at half-hour intervals. Figure 9 is a record of the centrifuging of a 1 per cent solution of electrolyzed, crystallized egg albumin at a pH of 7.4 in double-phosphate buffer. This particular experiment was performed at a temperature of 30° C. and a speed of 41,000 r.p.m. corresponding to a centrifugal force nearly 100,000 times that of gravity. The exposures were taken at half-hour intervals. The diffusion constant for the hemocyanin (Fig. 8) under the conditions of the experiment is 0.0014 cm.²/day, less than one seventieth that of the egg albumin in alkaline solution.

A reproduction is given in Figure 10 of the photographic record of a sedimentation equilibrium for a 1.9 per cent solution of egg albumin

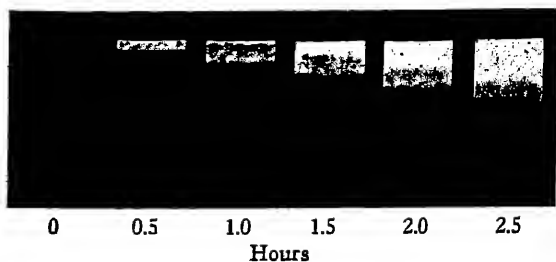


FIG. 8.—Sedimenting Hemocyanin (Chirnoagă).

subjected to a mean centrifugal force of 5.6×10^8 dynes (about 5,800 times the force of gravity) at 17° C. The series from right to left

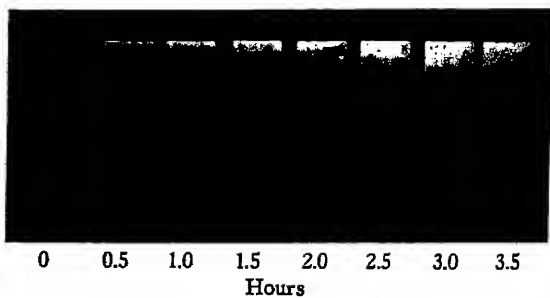


FIG. 9.—Sedimenting Egg Albumin.

includes a 40-second exposure at the start of centrifuging, followed by 20- and 40-second exposures after 39.5, 45.5 and 51 hours of centrifug-



FIG. 10.—Sedimentation Equilibrium of Egg Albumin.

ing, respectively. The last exposure to the left was taken after the solution was remixed at the end of the experiment.

An appreciable separation may be brought about by the centrifuging of molecules not separable by recrystallization. Figure 11 shows the concentration-distance curves that Lewis²⁷ obtained for a seventeen-year-old sample of phycoerythrin subjected to a centrifugal force approximately 100,000 times that of gravity. The dotted lines represent theoretical curves for a mixture of 70 per cent of molecules 208,000 in weight and 30 per cent, 208,000/8. A mixture containing two thirds of weight 208,000 and one-third 208,000/6 fits the conditions equally well. The curve *A* is the calculated curve for pure phycoerythrin of weight 208,000 after 80 minutes of centrifuging. Thus, by a procedure

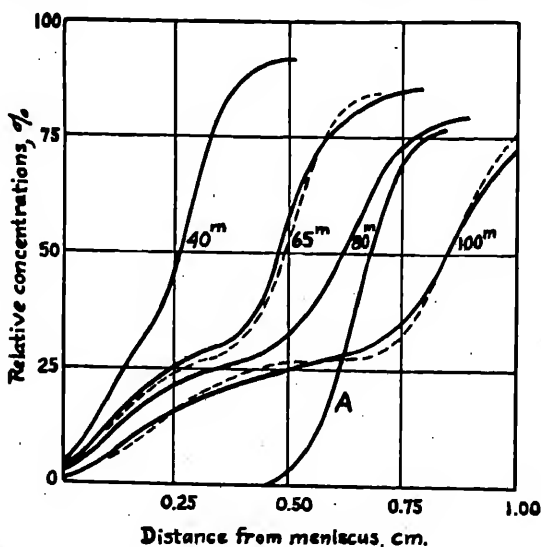


Fig. 11.—Centrifuging of Phycoerythrin (Lewis).

of alternately centrifuging and pipetting off the meniscus layers of a sedimented substance we should be able to effect the separation and analysis of a mixture that could not be fractionated or, more probably, not even be detected by other means.

Recently, several investigators, chiefly Troensegaard, Cohn, and Herzog²⁸ have been studying proteins in organic solvents—phenol and cresol. Troensegaard obtained a low molecular weight corresponding to a very simple molecule but Cohn questioned his results on account of the difficulty in drying the protein thoroughly. Since the values

²⁷ Svedberg and Lewis, *J. Am. Chem. Soc.*, 50, 534 (1928).

²⁸ Troensegaard and Schmidt, *Z. physiol. Chem.*, 133, 116 (1924); 167, 312 (1927); Herzog and Krahn, Herzog and Kobel, Herzog and Cohn, *Z. physiol. Chem.*, 134, 291, 296 (1924); 169, 305 (1927); Cohn and Conant, *Z. physiol. Chem.*, 159, 93 (1926).

obtained by the centrifugal method are not influenced by impurities of low molecular weight, the uncertainty as to the behavior of proteins in organic solvents can be cleared up very satisfactorily with the ultracentrifuge.

Table II shows the action of a Donnan potential on the sedimentation equilibrium of a 0.25 per cent solution of carbon monoxide-hemoglobin containing a small amount of combined acid (pH = 6.57). The solution was subjected to a centrifugal force 2700 times gravity at 16° C. The first two columns give the distances, x_2 and x_1 , from the axis of rotation, the next the logarithm of the ratio of the concentrations found at these distances, the fourth the normal values for $\log \frac{c_2}{c_1}$, and the fifth the ratio of the Donnan values to the normal values.

TABLE II. *Dialyzed Carbon Monoxide-Hemoglobin pH 6.57 Containing Combined Acid.*

Distances, cm.		Observed	Normal Values from $M=68,500$	Ratio of Observed to Normal Values
x_2	x_1	$\log \frac{c_2}{c_1}$	$\log \frac{c_2}{c_1}$	
4.68	4.63	0.0289	0.0422	0.685
4.63	4.58	.0262	.0418	.627
4.58	4.53	.0257	.0413	.622
4.53	4.48	.0265	.0409	.648
4.48	4.43	.0267	.0404	.661
4.43	4.38	.0261	.0400	.653
4.38	4.33	.0266	.0396	.672
4.33	4.28	.0267	.0391	.683
4.28	4.23	.0263	.0386	.682

Average 0.659

Concentration 0.25 gram per 100 cc.; partial specific volume $V = 0.747$ at 16°C.; density of solution $\rho = 0.9995$; $T = 289^\circ$; distance of outer end of cell from axis of rotation = 4.73 cm.; length of column of solution, 0.52 cm.; speed, 7300 r.p.m. ($\omega = 243.3 \pi$).

Since electrical work = centrifugal work - diffusional work²⁰ the electrical work under the conditions of the experiment is $1 - 0.659 = 0.341$ times as large as the centrifugal work. The centrifugal work required to move a mole of the hemoglobin from a distance 4.23 cm. to a distance 4.68 cm. from the axis of rotation is 2.0×10^{10} ergs; therefore the electrical work, $nF(\phi_{4.68} - \phi_{4.23}) = 0.682 \times 10^{10}$ ergs, where n is the valence or charge, F the Faraday constant and $(\phi_{4.68} - \phi_{4.23})$ is the potential difference between the concentrations of the protein present at the distances indicated, respectively 0.304 and 0.175 gram per 100 cc. The maximum potential difference which can arise from this separation is then 7.08 millivolts, if we make the assumption that hemoglobin behaves as if it were univalent near its isoelectric point.

²⁰ Cf. Tiselius, *Z. physik. Chem.*, 124, 457 (1926), for the theoretical equation.

The study of the interaction between proteins and dyestuffs might be mentioned as another possibility. Hewitt,³⁰ for example, has studied the action of phthalein dyes on serum albumin and egg albumin and finds that in acid solution the relation of moles dye to moles protein is approximately 30, thus leading him to conclude that chemical combination has occurred. However, if one determines the number of dye molecules required to form a monomolecular layer at the surface of the protein molecule, the figure turns out to be approximately 30 also. Therefore, should we consider this to be a combination or an adsorption? It would make a very interesting study to centrifuge protein solutions containing suitable amounts of dyes of varying complexity to ascertain whether the protein molecule takes up the same number of small dye molecules as it does of large, or whether sufficient are taken up in each case to form an adsorbed layer at the surface.

Information can be gained on the question of molecular symmetry also from an analysis of the diffusion constants obtained with the ultracentrifuge. For instance, hemoglobin appears to be an anisotropic molecule because its diffusion constant at 30° C. is 0.071 cm.² per day, as compared with 0.084 cm.² per day from the Einstein equation for a spherical particle. On the other hand, egg albumin, hemocyanin, and phycoerythrin seem to be nearly spherical or isotropic.

A complete study of the effect of light, heat, acid and alkali on the denaturation of proteins is possible. The change of hydration or adsorption relations with temperature can be followed. Deviations from the gas laws in concentrated solutions can be studied and activities of the protein and solvent determined. Studies can be carried out under conditions that would destroy osmometers as in concentrated alkalies or organic solvents. Finally, an insight could probably be gained of the inactivating property of a dyestuff like Congo red on toxins produced in botulism food poisoning, diphtheria, cobra venom, and the like.³¹

Thus, it is evident from the various examples cited above for the proteins, that the ultracentrifuge will have a very wide field of utility in the further advancement of our knowledge of the behavior and characteristics of the many natural and synthetic polymerized substances engaging our attention today, such as cellulose and its derivatives, rubber, and resins. Not alone for the lyophilic materials but also for the more lyophobic will the ultracentrifuge prove of value. Comparatively coarse particles in a very viscous medium³² (paint and varnish systems) and fogs or fumes can be investigated.

³⁰ *Biochem. J.*, 21, 1305 (1927).

³¹ Hanzlik and Butt, *Sci. News Letter*, 13, 246 (1928).

³² Nichols and Liehr, Colloid Symposium Monograph, Vol. 3, Chemical Catalog Co., Inc., New York, 1925, p. 268.

SUMMARY

1. The conditions have been outlined that must be fulfilled in order to obtain reliable and accurate data from the sedimentation of a substance in a centrifugal field of force.

2. The development of the ultracentrifuge has been traced from its inception to its present form capable of exerting an effect 100,000 times that of gravity on a solution.

3. A short discussion has been given of the thermodynamic quantities which it is possible to obtain through the establishment of an equilibrium between sedimentation and the counter diffusion in the centrifuge.

4. Some of the phenomena such as solvation and incipient gel formation have been mentioned which may be studied by the sedimentation velocity method.

5. A number of illustrations have been given from protein investigations carried out by means of the ultracentrifuge to indicate more concretely its wide field of usefulness.

Wilmington, Del.

THE STUDY OF HYDRATION CHANGES BY A VOLUME-CHANGE METHOD

BY H. A. NEVILLE AND H. C. JONES

INTRODUCTION

Previously reported experiments on the setting of plaster of Paris¹ indicated that the process occurs in two stages: (a) adsorption of liquid phase by solid, accompanied by *contraction* in volume, (b) exothermal chemical reaction between adsorbed liquid and adsorbent, accompanied by *increase* in volume—presumably due to heat evolved in the reaction. The setting of litharge-glycerin cement was shown to involve similar phenomena.²

The changes in volume noted in these experiments suggested that an accurate measurement of the variation in volume with time might be utilized to follow the course of such processes. The method seemed particularly applicable to processes involving hydration as, for example, the swelling of gels and the setting of cements.

The present report presents further data on the setting of plaster of Paris, but concerns principally the results obtained in the study of the setting of Portland cement. As the hydration or swelling of gelatin has been measured by various investigators and is recognized as a purely colloidal process, a few experiments with this material serve to show the utility of our apparatus for this purpose and to emphasize by a simpler analogy the mechanism of the setting of plaster of Paris and cement.

APPARATUS AND METHOD

The apparatus employed in these experiments, as shown in Figure 1, is essentially a simple dilatometer. However, in measuring the volume changes of a material which is liquid or mobile at the beginning of the experiment but solidifies during the period of measurement, lateral expansion or contraction in contact with rigid walls will introduce errors and may even deform the walls of the vessel. Hence, it is necessary to have the material completely surrounded by a viscous medium which

¹ Neville, *J. Phys. Chem.*, 30, 1037 (1926).

² *Idem*, 30, 1181 (1926).

will respond readily to the slightest contraction or expansion of the enclosed material. For this purpose a petrolatum mold, made by mixing vaseline and paraffin oil to the proper consistency proved satisfactory for the experiments with plaster of Paris and Portland cement. The mold was formed by means of a test-tube, a definite quantity of the material under investigation was then poured into the mold and the bottle portion of the apparatus was filled almost to the top with paraffin oil. The rubber stopper, supporting the capillary tube, was then inserted

without trapping any air bubbles and the screw clamps were adjusted until the oil stood at a definite height in the capillary. Readings were made on a millimeter scale placed behind the capillary.

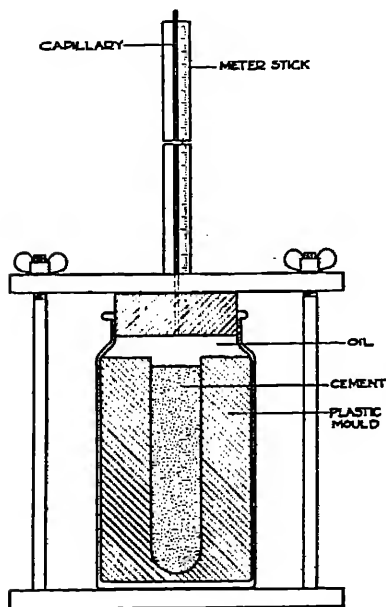


FIG. 1.—Apparatus.

The apparatus and all materials were always brought to a uniform temperature in a thermostat (27.6° C.) before each experiment, and the apparatus was immersed in the thermostat during the experiment. Because of the ease of construction and the compactness of the apparatus, several of them were used concurrently in the same thermostat. Results obtained with these different dilatometers will, of course, be comparable quantitatively only if the capillaries have the same bore and if the same quantity of reaction mixture is used in each mold. The sensitivity of the apparatus can be increased by using a capillary having a smaller bore or by

using a larger sample of the mixture in the mold.

A dilatometer has been described by Hampton³ and recommended for measuring the hydration of colloids. A somewhat similar piece of apparatus was used by Svedberg in measuring the swelling of gelatin.⁴ Both of these forms are limited to the use of test samples which are already rigid before they are placed in the apparatus.

The measurement of the volume changes which occur in the setting

³ *Science*, 63, 49 (1926).

⁴ *J. Amer. Chem. Soc.*, 46, 2673 (1924).

and hardening of Portland cement is obviously of great practical importance. Many studies of this feature have been made, principally by investigators whose interests have been entirely practical. The usual method has been the measurement of linear changes in set cement test-blocks, with little effort to maintain constant conditions of temperature and humidity. The apparatus described in this paper offers advantages for this purpose in that conditions are rigidly controlled, measurements can begin immediately after mixing, and the measurements are cubical in value.

RESULTS AND DISCUSSION

PLASTER OF PARIS

In these experiments 40 grams of plaster of Paris were mixed with 30 cc. of water, stirred for 1 minute, and poured into the mold. The temperature-time data were obtained as previously reported.¹ Figure 2 shows the correspondence between temperature rise and volume change

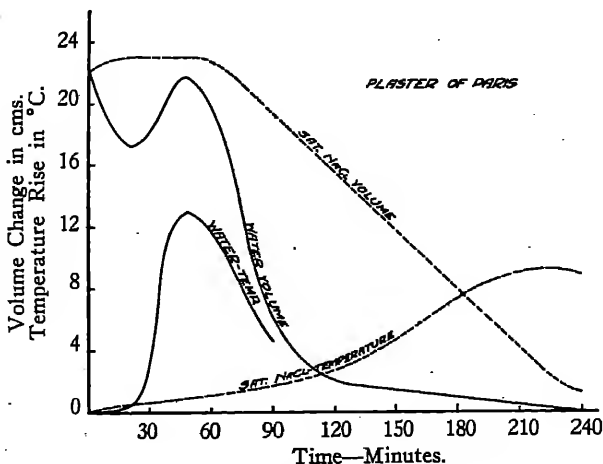


FIG. 2.—The Hydration of Plaster of Paris.

in the setting of plaster of Paris. The minimum at 20 minutes on the volume curve corresponds in point of time to the "initial set." At this time the plaster cast has a dry appearance and resists indentation; but on the temperature curve there has been no appreciable rise, and hence the reaction



has not occurred. Beyond this point the temperature rises rapidly and the volume increases correspondingly, due to thermal expansion. That this is the cause of the expansion is proved by the coincidence of the maxima on the two curves at 45 minutes and the subsequent contraction as the temperature falls. However, the plaster of Paris contracts to a volume considerably smaller than it had at the time of "initial set," or when the exothermal reaction began. This indicates that the hydration process which caused the initial contraction has really been occurring throughout the whole period, though it was masked for a time by the thermal expansion. The plaster of Paris reaches a constant volume only after 4 hours.

When plaster of Paris is mixed with a saturated solution of sodium chloride instead of with water, the hydration process is retarded. This is shown on the temperature curve by the fact that the "initial set" requires about 2 hours and the maximum temperature is attained only after 3.5 hours. On the volume curve the contraction is likewise greatly retarded, and no expansion is observed since the heat is evolved so slowly that it is dissipated in the thermostat. The hydration of plaster of Paris is accelerated by dilute solutions of most salts.

PORTLAND CEMENT

A commercial Portland cement was used. The stock was obtained fresh from the cement plant and was carefully protected from exposure to the atmosphere. A mixture of 40 grams of cement with 20 cc. of water (or solution) was used in these experiments. This mixture was stirred for 3 minutes before it was poured into the mold. It was found that increasing the quantity of water to 30 cc. or extending the time of stirring to 6 or 9 minutes would not cause an appreciable difference in the results.

The thermal effect in this mixture, determined as in the case of plaster of Paris, produced a maximum rise of 2° C. in 12 minutes.

Figure 3 includes some volume change curves for Portland cement mixed with water and with the solutions indicated on the curves. The *initial expansion*, noted particularly on the water curve, is attributed to purely thermal effects which may include: the heat of wetting (Pouillet effect); the heat of hydration and solution of free lime; the heats of hydration and solution of burned gypsum products, as about 2 per cent of gypsum is added in the manufacture of Portland cement; the heat of reaction from the hydrolysis of certain components of the cement. The amount of free lime in the cement used was found by the

method of Leach and Bogue⁵ to be 0.67 per cent. It can be shown by calculation that this percentage of free lime is too small to account by itself for the total heat evolved.

The subsequent contraction of the cement-water mixture may be explained as quite analogous to that observed with plaster of Paris. It is caused by the adsorption of water by the finely divided cement particles and the hydration of the particles to form a gel.

The effect of electrolytes on the setting of Portland cement is quite marked. In some cases the mixture becomes so stiff that it cannot be poured after it is stirred for 3 minutes. Dilute solutions of simple salts

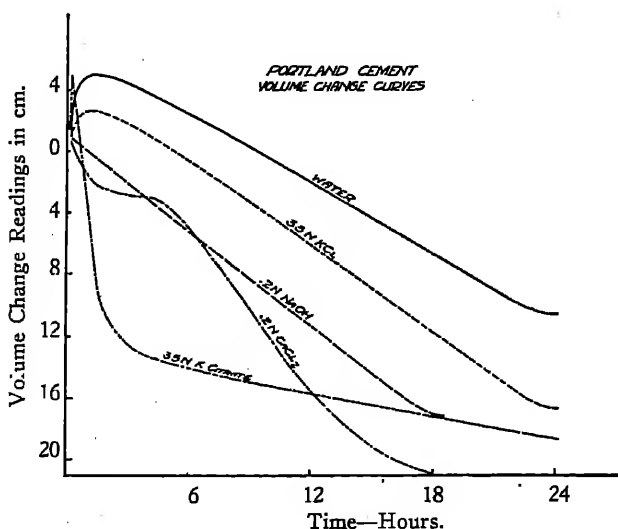


FIG. 3.—The Hydration of Portland Cement.

in equivalent concentrations were studied in an attempt to arrange the anions and cations in series with respect to their influences on the setting of cement. All these solutions showed an appreciable effect, but their differences were so slight that it did not seem justifiable to place the ions in any definite order on this basis.

The curve shown in Figure 3 for 3.5 *N* KCl, which is an almost saturated solution, may be considered as representative of the effects of the more concentrated solutions of simple salts.

The curve for 0.2 *N* NaOH and the curve for 0.2 *N* CaCl₂ show no initial increase in volume. This can be explained on the assumption

⁵ *J. Ind. Eng. Chem.*, 18, 739 (1926).

that the hydration causing the contraction sets in so rapidly that the expansion due to thermal effects is more than compensated. Since calcium chloride is sometimes added in concrete mixtures, the study of the effect of this salt is of practical significance. The theories offered to explain the effect of calcium chloride in accelerating the hardening of Portland cement are reviewed by Weiser.⁶ In practice, two factors seem to be involved: the hygroscopic nature of calcium chloride and its effect in decreasing the pH, thus accelerating the hydrolysis of the components of the cement.

The behavior of the mixture of cement with a 3.5 *N* solution of

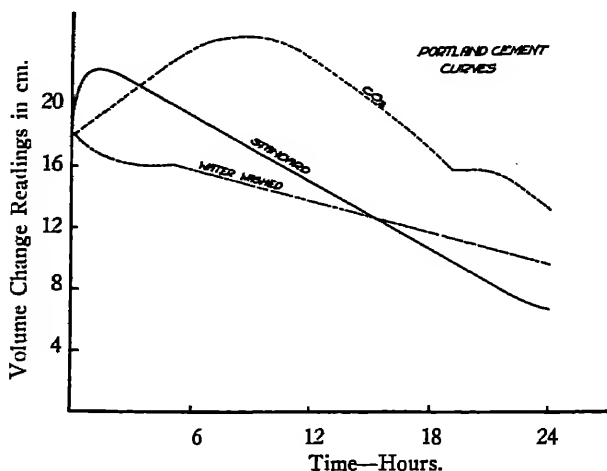


FIG. 4.—Volume Changes of Treated Portland Cement.

potassium citrate is interesting from the colloid viewpoint. The citrate ion is known to be "capillary active" or highly adsorbed by colloidal matter. Through its influence the volume change was accelerated to such an extent that the mixture had completed in 3 hours the same phases that required 24 hours in the cement-water mixture.

Samples of dry cement were exposed to carbon dioxide gas to convert the free lime to calcium carbonate. It was thought that at least part of the initial increase in volume of cement-water mixtures was due to the heat of solution and hydration of free lime, and that if the free lime were eliminated, the initial expansion would be decreased. The labelled curve in Figure 4 shows that this did not occur, but that the whole process of hydration was considerably retarded.

⁶ "The Hydrous Oxides," New York, McGraw-Hill Book Co., 1926, p. 393.

It is probable that carbon dioxide gas was adsorbed by the cement powder and when this material was subsequently mixed with water, the carbon dioxide reacted chemically with the cement modifying the hydration capacity of the latter. Thus the known changes in the rate of setting of cements which have been exposed to the atmosphere for a considerable time may be partly attributed to the effect of adsorbed carbon dioxide.

Another curve in Figure 4 shows the effect of washing the cement with water. Samples of cement were shaken with an excess of water for 5 minutes, allowed to settle for 5 minutes, and the clear supernatant liquid was siphoned off. It was thought that some of the free lime and gypsum products might be removed by this means. The absence of any initial expansion of the mixture indicates that this has probably been accomplished, though other effects may have been involved.

GELATIN

A high grade commercial gelatin in granular form was used in these experiments. No mold was employed in the dilatometer and the

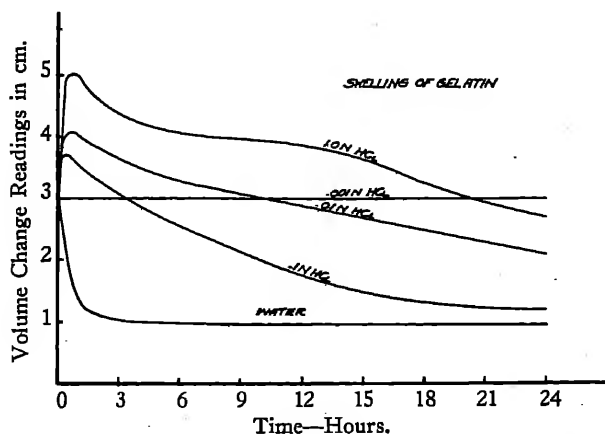


FIG. 5.—The Swelling of Gelatin in Concentrations of Acid.

water served as the indicating liquid in the capillary. The dry gelatin was placed in the bottle which was then filled with water or an acid solution. Five grams of gelatin were used and the capacity of the dilatometer was 150 cc.

The curves in Figure 5 represent the results obtained with gelatin.

The so-called swelling of gels is, of course, accompanied by a net decrease in volume when the liquid is taken into account. The gelatin placed in 0.001 *N* HCl showed no volume change whatever, although it became uniformly dispersed and set to a weak gel. A determination of the pH of this gel by means of indicators gave a value of 4.7 which is the isoelectric point of gelatin. The maximum swelling was obtained with pure water and with 0.1 *N* HCl. The pH values of these two media are on opposite sides of the isoelectric point. The stronger and weaker solutions of hydrochloric acid (*N* and 0.01 *N*) produced less swelling than the 0.1 *N* solution. Thus the results agree with the familiar curve relating swelling of gelatin to pH value.⁷ For this comparison the equilibrium values at 24 hours are taken.

It is noted that the acid solutions, except the isoelectric concentration, caused an initial increase in volume. This is probably due to exothermal chemical reaction between the acid and the gelatin. The gelatin in 0.01 *N* acid showed only a slight initial rise on the curve because its hydration or swelling was occurring so rapidly that the contrary effect is partly compensated. The quantity of gelatin used in these experiments did not form a gel with any of the acid solutions as it did with pure water.

THE MECHANISM OF HYDRATION PROCESSES

In Figure 6 the striking similarity of the three hydration processes here discussed is emphasized by plotting the three volume-change curves to different scales. If the swelling of gelatin in pure water may be assumed to be a strictly colloidal process consisting in the adsorption of water to form a hydrated material of gel structure but no definite hydrates, the setting of plaster of Paris and the setting of Portland cement may be considered as quite analogous phenomena.

In the two latter cases, however, there is an apparent complication caused by the incidental occurrence of exothermal chemical reactions to produce chemical substances not present in the original material. The humps above the broken portions of the curves are caused by the thermal effects accompanying these reactions. This same phenomenon is noted when gelatin swells in acid solutions where the pH is less than 4.7. The reactions which produce these new substances are surface reactions, occurring in adsorption films; the products are in the colloidal condition and readily form gels. In the case of plaster of Paris a gel of hydrated calcium sulfate is produced in which crystals of gypsum grow as the gel ages.

⁷ Cf. Loeb, "Proteins and the Theory of Colloidal Behavior," New York, McGraw-Hill Book Co., 1922, p. 76; or Kruyt, "Colloids," New York, Wiley & Sons, Inc., 1927, p. 229.

Authorities are generally agreed that a gel of tri-calcium aluminate is formed in the initial period of the setting of Portland cement. Other gelatinous substances may be produced by hydrolysis, such as mono-calcium silicate, silicic acid, the hydrous oxides of aluminum, calcium, iron and magnesium. It has been demonstrated by White that set Portland cement behaves as a gel. In this paper, "Hydrated Portland

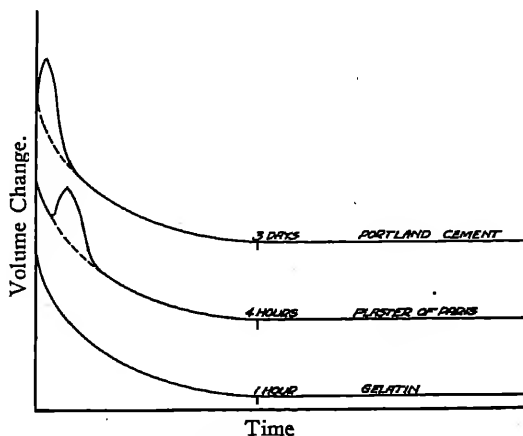


FIG. 6.—Related Hydration Curves.

Cement as a Colloid,"⁸ he states that it exhibits the characteristic shrinkage and swelling for at least 20 years with changing conditions of humidity.

It is planned to study the separate constituents of Portland cement by the method outlined in this paper.

SUMMARY

1. A dilatometer having certain new features is described. This apparatus is suitable for measuring the variation in volume of a material which changes from mobile to solid condition during the experiment.

2. The setting of plaster of Paris, as studied by this means, is related to measurements of temperature rise in the same process.

3. The method is applied to the setting of Portland cement, and the influence of various added substances upon the rate of setting is shown.

⁸ "Colloid Symposium Monograph," Vol. 5, New York, The Chemical Catalog Co., Inc., 1927, p. 349.

4. The swelling of gelatin in solutions of different acidity is measured.

5. The mechanism of the hydration process is shown to be strikingly similar in the following cases: the swelling of gelatin; the setting of Portland cement; the setting of plaster of Paris.

*Lehigh University,
Bethlehem, Pennsylvania.*

ADSORPTION OF IONS AND THE PHYSICAL CHARACTER OF PRECIPITATES

BY HARRY B. WEISER AND G. E. CUNNINGHAM

The first systematic investigation of the form in which substances precipitate from solution was made by von Weimarn.¹ He calls attention to a number of different factors on which precipitation depends: the solubility of the substance, the latent heat of precipitation, the concentration at which the precipitation takes place, the normal pressure at the surface of the solvent, and the molecular weights of the solvent and solute. He points out the impossibility of taking all these factors into account and simplifies the problem by considering but two of the factors: the solubility of the precipitating substances and the concentration at which the precipitation begins. The process of condensation (precipitation) is considered as taking place in two stages: the first stage, in which the molecules condense to invisible or ultramicroscopic crystals; and the second, which is concerned with the growth of particles as the result of diffusion. The velocity of the condensation at the important first moment of the first stage of the process is formulated thus:

$$W = K \frac{\text{condensation pressure}}{\text{condensation resistance}} = K \frac{Q - L}{L} = K \frac{P}{L} = KU \quad [1]$$

in which W is the initial rate of precipitation, K a constant, Q the total concentration of the substance that is to precipitate, L the solubility of coarse crystals of the substance, $Q - L = P$ the amount of supersaturation. The ratio $P/L = U$ is the percentage supersaturation at the instant precipitation begins. To take care of other factors which may enter into the process, von Weimarn introduces a "variable multiplier," J , and the equation becomes:

$$W = KJ \frac{Q - L}{L} \quad [2]$$

The velocity of the second stage of the process is given by the Nernst-Noyes equation:

$$V = \frac{D}{S} \cdot O \cdot (C - I) \quad [3]$$

¹ *Kolloid-Z.*, 2, 199, 230, 275, 301, 326; Supplement 2, LII; 3, 89, 282 (1908); 4, 27 (1909); "Grundzüge der Dispersoidchemie" (1911); "Zur Lehre von den Zuständen der Materie" (1914).

where D is the diffusion coefficient, S the thickness of the adherent film, O the surface, C the concentration of the surrounding solution and l the solubility of the disperse phase for a given degree of dispersity. $C - l$ may be termed the absolute supersaturation.

By the aid of these equations, several facts are interpreted. It will be seen that the velocity of precipitation depends not alone upon the supersaturation P , but upon the percentage supersaturation P/L . Thus, with a given value of P (say a few grams per 100 cubic centimeters), a very soluble substance, such as sodium chloride, will deposit nothing at first and finally a few crystals may form; but with the same value of P an almost insoluble substance, such as alumina or silver chloride, will give an immediate gelatinous or curdy precipitate. The difference is, that the velocity of the precipitation is much smaller in the first case than in the second. On the other hand, if sodium chloride is formed by the interaction of sodium ethylate or sodium thiocyanate and hydrochloric acid in a mixture of ether and amyl alcohol, in which sodium chloride is practically insoluble, the precipitate is curdy like that of silver chloride.

While the value of P is not in itself of primary importance in determining the form of the precipitate, it is not without influence, since quite different results are obtained, depending on whether a given value of U is obtained by a large P or a small L . In the first case a large amount of disperse phase must be produced, and in the second very little. Hence, if U is large, the former will, in general, give a gelatinous precipitate or jelly, and the latter a larger number of highly dispersed particles—a sol.

Von Weimarn recognized that the velocity W of the first stage of precipitation cannot be measured in actual practice, and that, in many cases especially interesting in the synthesis of colloid systems, the velocity U of the growth of the particles cannot be determined. In due time, therefore, he introduced a specific coefficient called the "precipitate form coefficient" or "dispersity coefficient," N , which is given by the expression:

$$N = \frac{P}{L} \cdot K_{ab} \cdot K_{cd} \cdot K_{bd} \cdot K_{ac} \cdot Z \quad [4]$$

in which P/L is the percentage supersaturation as in the velocity equation, Z the viscosity, and K_{ab} , K_{cd} , etc., represent the "physical and chemical association" of the substances AB , CD , etc., which enter into the reaction: AB (in solution) + CD (in solution) = AC (precipitate) + BD (in solution). The significance of "physical association" is known but it is not clear what von Weimarn means by "chemical association."

This makes no difference however, for the several factors are neglected and N is set down equal to P/L , that is,

$$N = \frac{P}{L} \quad [5]$$

or better,

$$N = J \frac{P}{L} \quad [6]$$

in which J has the same significance as in equation (2).

Now if N is taken as approximately equal to P/L , as von Weimarn first assumed, then for the different substances x , y , and z ,

$$N_x = \frac{P_x}{L_x}; \quad N_y = \frac{P_y}{L_y}; \quad \text{and} \quad N_z = \frac{P_z}{L_z}.$$

If the character of the precipitate is to be the same in each case, irrespective of the chemical nature of the salt; in other words, if

$$N_x = N_y = N_z$$

then

$$\frac{P_x}{L_x} = \frac{P_y}{L_y} = \frac{P_z}{L_z} \quad [7]$$

This is the simplest expression for von Weimarn's Law of Corresponding States for the Precipitation Process, which says that under corresponding conditions of precipitation the mean magnitude (expressed in gram molecules) of the crystals of substances capable of precipitation will be the same. In the form given in equation (7) the so-called law can hardly be regarded as a first approximation, even with substances that are related chemically. This is illustrated by the observations of Buchner and Kalff² recorded in Table I. L is the solubility of the several salts and N is calculated from the concentrations of the solutions employed, using the expression $N = P/L$.

TABLE I. *Physical State of Precipitates.*

Salt	L	N	State of Precipitate
CaF ₂	4×10^{-4}	3,400	Jelly
BaF ₂	18×10^{-3}	75	Jelly
CaSO ₄	3×10^{-2}	140	Jelly
BaSO ₄ ..	2×10^{-2}	100,000	Jelly
AgCl	1×10^{-5}	700,000	Colloidal, unstable
AgBr	7×10^{-7}	8,000,000	Colloidal, unstable
AgI	1.5×10^{-8}	30,000,000	Jelly, unstable
PbI ₂	4.8×10^{-3}	360	Colloidal

Considering the first four salts, it will be seen that the nature of the precipitate is the same although the value of N varies between 75

² *Rec. trav. chim.* 39, 135 (1920).

and 100,000. Von Weimarn obtained well-defined crystals of barium sulfate at values of N varying between 50 and 20,000. The law of corresponding states in the simplest form would require that, under the conditions recorded in the table, CaSO_4 , CaF_2 , and BaF_2 should give crystalline precipitates instead of transparent jellies. Considering next the silver halides: If von Weimarn's law in its simplest form held, these salts should give jellies more stable than BaSO_4 under the conditions used; but, instead, they form colloidal solutions which later come down in flocks of an entirely different physical character from the barium sulfate jelly. Finally, the small value of N for PbI_2 would lead one to expect the formation of a definitely crystalline precipitate under the conditions employed. Actually, a jelly results.

Von Weimarn's³ explanation of these discrepancies is, of course, that the law of corresponding states for the precipitation process is not the simple expression

$$\frac{P_x}{L_x} = \frac{P_y}{L_y} = \frac{P_z}{L_z} \dots$$

but

$$J_x \cdot \frac{P_x}{L_x} = J_y \cdot \frac{P_y}{L_y} = J_z \cdot \frac{P_z}{L_z} \dots$$

in which J_x , J_y , and J_z are specific variable multipliers, the value for any substance being "the product of all other factors (in addition to P/L) which influence the crystallization process. These values must be expressed by abstract numbers such that the values of P/L are equivalent."³ In other words, von Weimarn's equation for his so-called law becomes quantitative and generally applicable by putting in "variable multipliers," handy waste-baskets, as it were, into which are thrown all the variable factors known or unknown which have not been evaluated.

While facts may be expressed fairly accurately by means of such flexible formulas, it is doubtful whether anything is gained scientifically by regarding formulations of this kind as quantitative representations of natural laws. Von Weimarn evidently thinks so, but his opinion is not shared generally. Thus, Bancroft⁴ prefers to discard the formulas altogether and state the whole thing from a different point of view. He points out that the mean size of the crystals is determined by the total amount of material crystallizing and the number of nuclei. The really important thing, therefore, is the number of nuclei which are formed under any given conditions. It is contended, very properly, that factors other than percentage of supersaturation influence the number of nuclei formed. Thus, the specific nature of the substance, stirring and

³ *Kolloidchem. Beihefte*, 18, 48 (1923).

⁴ *J. Phys. Chem.*, 24, 100 (1920).

temperature have a profound effect on nucleus formation, and adsorption exerts a marked influence on the growth of particles.⁵ Freundlich⁶ likewise does not believe that the separation of a solid phase is generally and uniformly regulated by its solubility and the supersaturation prevailing: "What is known concerning the extraordinary sensitiveness to foreign substances of the velocities of formation of nuclei and crystallization makes it *a priori* improbable, and experience has not confirmed this theory."

The effect of adsorption on the physical character of precipitates is considered in the following way by von Weimarn:⁷ At constant $U = P/L$, foreign molecules have the same effect as greatly increasing U . The reason is that, during the growth of grains, the foreign matter keeps the reacting solutions away, so that not far from a given grain a local supersaturation results in the formation of a new grain. This would not happen in the absence of foreign molecules.

As would be expected, von Weimarn⁸ includes the variable adsorption factor in his "variable multiplier": "According to my theory, the adsorption factor at constant P/L acts in the same way as simply increasing P/L ; that is, the precipitate is more highly dispersed. Now I have included the adsorption factor in the variable J of the precipitate form coefficient, $N = J.(P/L)$. It follows, therefore, that J is increased by adsorption. If, however, P/L remains constant it follows that the value of N must likewise increase. A larger value of N corresponds to a higher degree of dispersion of the precipitate."

This categorical way of disposing of the effect of adsorption on the physical character of precipitates is not helpful. Von Weimarn's view is that the foreign substance always forms a protective film around the particles and thereby prevents their growth. This is apparently what takes place when precipitation occurs in the presence of a strongly adsorbed protective colloid such as gelatin. However, the effect on the physical character of a precipitate, produced by the adsorption of the solvent and of ions of varying charge and degree of hydration, cannot be disposed of so simply as von Weimarn would lead one to expect. Any substance should form a gel provided a suitable amount of highly dispersed substance is precipitated, and, provided the particles adsorb the dispersion medium very strongly.⁹ The amount of the dispersed phase that must be present in a given volume to form a jelly will depend on the size and orientation of the particles and the extent to which they adsorb the dispersing liquid, water in most cases. Now it is too well known to need comment that some substances adsorb water

⁵ Weiser, *J. Phys. Chem.*, 21, 314 (1917).

⁶ "Kapillarchemie," 631 (1922).

⁷ "Grundzüge der Dispersoidchemie," 97 (1911).

⁸ *Kolloidchem. Beihefte*, 18, 68 (1923).

⁹ Weiser, "The Hydrous Oxides," McGraw-Hill Book Co., New York, 1926, p. 26, 27.

more strongly (that is, are much more hydrous) than others, so that at the same degree of dispersity some substances will form gels while others will not. This specific capacity of the particles to adsorb the solvent is altogether independent of their size and the rate of precipitation. In many instances, this is of far more importance than the rate of precipitation in determining the form of the precipitate. A notable example is the case of manganese arsenate, which can be made to form a stiff jelly by mixing very dilute solutions of potassium arsenate and manganese sulfate. The value of L for the precipitate is so large that precipitation is slow and quantitative precipitation is impossible. $P/L = N$ is very small, and yet a typical transparent jelly results.¹⁰

Von Weimarn would take care of the variation in the adsorption of the solvent by the particles by putting it into the J of the expression $N = J.(P/L)$, and so make the calculated values of N fit the facts. There is, of course, no objection to doing this, but it is difficult to see what is gained by such a procedure.

The peptizing action of adsorbed ions may have a marked effect on the physical character of a precipitate. Thus, the analyst knows that barium sulfate, formed in ordinary analytical procedures, comes down very much finer when precipitated with barium chloride in excess than with sulfuric acid in excess. This happens notwithstanding the fact that barium ion and sulfate ion are adsorbed about equally, and yield positive and negative sols, respectively, when the precipitation is carried out under suitable conditions. Finer crystals are obtained from potassium sulfate solution than from sulfuric acid. The explanation of this behavior is as follows: In addition to adsorbing its own ions strongly, barium sulfate adsorbs hydrogen ion more strongly than most cations. When sulfuric acid is treated with barium chloride in excess, the precipitate tends to come down in a finely divided state because of the peptizing action of the relatively strongly adsorbed barium and hydrogen ions. It would also come down in a finely divided state from a solution containing sulfuric acid in excess were it not that the strongly adsorbed hydrogen ion neutralizes the peptizing action of the sulfate ion. From potassium sulfate solution it comes down finely divided since potassium ion is not strongly absorbed.¹¹

That the effect of foreign material on the physical character of a precipitate may not be due chiefly to increasing the number of points of crystallization, is well illustrated in the case of sulfur, which is thrown down from the sol in a variety of conditions in the presence of different electrolytes. Thus, Stingl and Morawski¹² showed that potassium and barium salts precipitate sulfur in a plastic form, while calcium mag-

¹⁰ Weiser and Bloxson, *J. Phys. Chem.*, **28**, 26 (1924).

¹¹ Weiser, *J. Phys. Chem.*, **21**, 314 (1917).

¹² *J. prakt. Chem.* (2), **20**, 76 (1879).

nesium, and sodium salts give flocculent sulfur. Odén¹³ states that sulfur is thrown down from the sol as a hard precipitate with potassium salts, fine-grained with copper sulfate, plastic with barium salts, fluid with hydrochloric acid and slimy with other salts. Since the physical character of sulfur thrown down in the presence of different electrolytes shows such marked variations, it seemed to furnish a satisfactory substance for studying the general effect which the adsorption of ions may have on the physical character of precipitates. The results of this study are reported in the next section.

EXPERIMENTAL

FORMATION OF SULFUR SOL

Colloidal solutions of sulfur are formed by the interaction of solutions of hydrogen sulfide¹⁴ or alkali sulfides¹⁵ and sulfurous acid; and by the decomposition of thiosulfate with hydrochloric acid¹⁶ or sulfuric acid.¹⁷ A sol is formed also by pouring an alcoholic solution of sulfur into water.¹⁸ In the latter case the dispersion is relatively unstable, the particles agglomerating and settling out in a short time.

The sols used in this investigation were prepared by an adaptation of the method used by Sobrero and Selmi,¹⁴ namely, by passing hydrogen sulfide and sulfur dioxide simultaneously into a saturated solution of sulfur dioxide. By dissolving various amounts of sulfur dioxide in water and then saturating with hydrogen sulfide, Odén found that a high initial concentration of sulfur dioxide favors the formation of finely divided particles, whereas a low initial concentration of the same gas favors the formation of non-peptizable clumps. Accordingly, in order to obtain large quantities of colloidal sulfur, the water was first saturated with sulfur dioxide and the excess was then maintained throughout the reaction by passing the theoretical amount of sulfur dioxide for the reaction, $\text{SO}_2 + 2\text{H}_2\text{S} = 2\text{H}_2\text{O} + 3\text{S}$, into the water simultaneously with the hydrogen sulfide. By this method it was possible to carry on the reaction for several hours without changing the reaction medium. After the sol became highly concentrated, sulfur settled out as a yellow mass which was readily peptized by shaking with water. The constancy of the conditions of formation gave particles of a much more

¹³ "Der kolloide Schwefel," 134, 157 (1912).

¹⁴ A. Berthollet, *Ann. chim.*, 25, 233 (1798); Sobrero and Selmi, *Ann. chim. phys.* (3), 28, 210 (1850).

¹⁵ Wackenroder, *Archiv Pharm.*, 48, 272 (1840).

¹⁶ Engel, *Compt. rend.*, 112, 868 (1891); Raffo, *Kolloid-Z.*, 2, 358 (1908).

¹⁷ Odén, *Z. physik. Chem.*, 80, 709 (1912); "Der kolloide Schwefel" (1912); *Novo Acta (Upsala)*, 3, No. 4 (1913).

¹⁸ Von Weimarn and Malyshev, *J. Russ. Phys. Chem. Soc.*, 42, 484 (1911); *Kolloid-Z.*, 8, 216 (1911).

uniform degree of dispersion than could be obtained by strict adherence to Odén's method.

The characteristic hydrophilic properties of Odén's sulfur sol are attributed by Freundlich¹⁹ to the presence of pentathionic acid in the micelles. This conclusion is based on the observation that the sols have different properties if obtained under conditions such that the pentathionic acid cannot form. There seems to be no good reason for believing that the only thionate present in the micelles is the pentathionate, since Debus²⁰ showed that the mixture formed by the interaction of sulfur dioxide and hydrogen sulfide contains, in addition to colloidal sulfur and pentathionic acid, di-, tri-, tetra-, and probably hexa-thionic acids.

For the preliminary macro-observations on the physical character of the precipitated sulfur, the sol prepared as described above was purified by finally saturating the reaction liquid with hydrogen sulfide and then boiling out the excess gas. Any non-peptizable sulfur was removed by filtering through a fine filter paper. The sol obtained in this way contained nothing except the products of the reaction by which it was formed, and required no dialyzing. Moreover, it was quite stable, a part of the colloidal particles remaining suspended for more than two years. On the other hand, due to the decrease in the concentration of sulfur dioxide at the end of the reaction, the particles showed considerable variation in size, and for the microscopic and submicroscopic investigations, sols having particles of approximately uniform size were preferable.

The preparation of such monodisperse sols was readily accomplished, since the sol thrown down by sodium chloride is reprecipitated by washing with water, and since the smaller particles are more stable and require a higher concentration of salt to cause coagulation.²¹ The following procedure was employed: The original sol was completely coagulated by the smallest possible concentration of sodium chloride, the coagulum collected by the aid of the centrifuge and reprecipitated with water. This process was repeated several times, until the supernatant liquid after coagulation gave no test for sulfite or sulfate with barium chloride. This sol was split into fractions by adding sodium chloride stepwise to it and separating the sulfur not coagulated at each step. In order to ensure complete fractionation, it was necessary to repeat the treatment with each concentration of sodium chloride several times. Each fraction was then concentrated by precipitating with sodium chloride and reprecipitating with a smaller volume of water. The same

¹⁹ *Kolloidchem. Beihefte*, 16, 234 (1912); "Colloid and Capillary Chemistry," 618 (1927).

²⁰ *Chem. News*, 57, 87 (1888).

²¹ Odén, *Z. physik. Chem.*, 78, 682 (1911).

differential fractionation concentrations of sodium chloride recommended by Odén and given in Table II were employed.

TABLE II. *Differential Fractionating Concentrations of Sodium Chloride for Sulfur Sol.*

Fraction Number	Normal Concentration of Sodium Chloride	
	Which Coagulates	Which Does Not Coagulate
1.....	0.25	...
2.....	0.20	0.25
3.....	0.16	0.20
4.....	0.13	0.16
5.....	0.10	0.13
6.....	0.07	0.10
7.....	0.00	0.07

After the several fractions had been concentrated as described above they were dialyzed for two to three weeks, until the dialysate showed no residue on evaporation and gave no test for chlorides. The first dialyses were done with parchment membranes, which gave trouble because of the tendency of the particles to diffuse through. A similar difficulty was reported by Nevinsky.²² Later dialyses were made with "cellophane" membranes, which proved to be entirely satisfactory.

COAGULATION OF SULFUR SOL BY ELECTROLYTES, AND THE FORM OF THE PRECIPITATE.

The precipitation values of a series of electrolytes for the sol were determined using a mixing apparatus consisting of a small test tube sealed inside a larger one.²³ Ten cubic centimeters of sol was placed in the inner compartment of the apparatus and a measured amount of standard electrolyte, diluted to 10 cc., in the outer compartment. The stopper was inserted in the mixer which was shaken vigorously, thus ensuring rapid and uniform mixing. The mixture was then poured into a Pyrex test tube and allowed to stand for 15 minutes, at the end of which time it was centrifuged for 3 minutes at 2000 r.p.m. By trial, the concentration of electrolyte was determined which was just sufficient to leave a clear supernatant liquid. At the same time, an amount of electrolyte slightly in excess of the precipitation concentration was added to the sol, and the nature and physical character of the precipitate was observed after allowing the mixture to stand 15 minutes and then shaking. The results are given in Table III.

Since the descriptions of the precipitates obtained with different precipitating ions are of necessity somewhat indefinite, some repre-

²² *Berl. klin. Wochschr.*, 45, 1833 (1908).

²³ Weiser and Middleton, *J. Phys. Chem.*, 24, 48 (1920).

sentative photographs (actual sizes) were made of the clumps which constitute the precipitates. The precipitations were carried out in the mixing apparatus, and the mixture was then poured into a flat-bottomed cell made by cementing a short length of hard rubber tube (2.5 inches inside diameter) onto an optically plane glass plate. A camera was

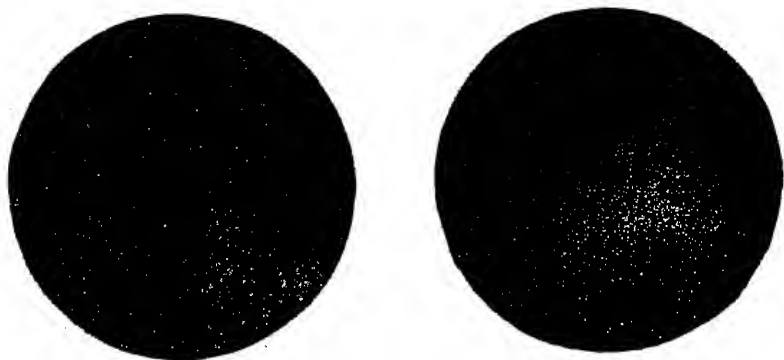
TABLE III. *Coagulation of Sulfur Sol by Electrolytes and the Physical Character of the Coagulum.*

Electrolyte	Precipitation Concentration, Milliequivalents per cc.	Physical Character of the Precipitate
HCl	0.5	Slimy; reversible
LiCl	0.55	Gelatinous; reversible
NaCl	0.25	Gelatinous; reversible
KCl	0.12	Plastic flocks; irreversible
CsCl	0.12	Plastic flocks; irreversible
CuCl ₂	0.012	Gelatinous; reversible on repeated washing
MgCl ₂	0.015	Granular; reversible on repeated washing
ZnCl ₂	0.015	Granular; reversible on repeated washing
CaCl ₂	0.008	Granular; partly reversible
SrCl ₂	0.006	Curdy; irreversible
BaCl ₂	0.0076	Plastic; irreversible
FeCl ₃	0.00045	Curdy; almost entirely reversible
AlCl ₃	0.00035	Curdy; almost entirely reversible
H ₂ S	(Saturated)	Only a small amount of slimy precipitate formed in 4 hours; almost entirely re- versible.
NaOH	0.15	Curdy to plastic; slightly reversible.

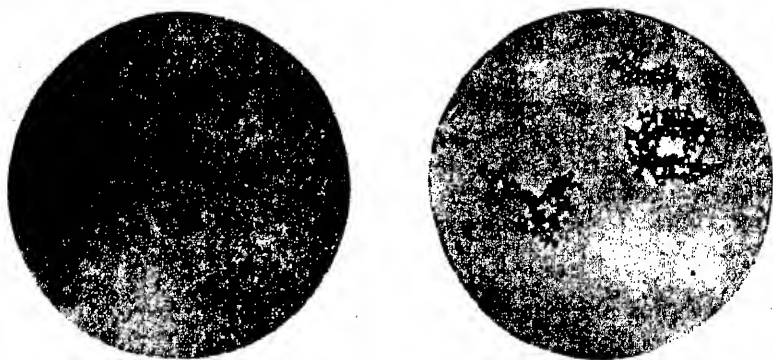
mounted above the cell and the precipitates were photographed on orthochromatic film by light filtered through a 1.5-inch thickness of saturated picric acid solution and reflected upward through the bottom of the cell. In each case a photograph was made (*a*) immediately after pouring the mixture into the cell and (*b*) after agitating the cell to further agglomerate the coagulum. The results are shown in Figure 1.

The fact that the character of the precipitate is not determined by the rate of precipitation was shown in the following way: Ten cubic centimeters of sol was placed in a test tube, and 10 cubic centimeters of electrolyte solution containing enough NaCl or BaCl₂ for exact precipitation was poured into a cellophane cup partly immersed in the sol. After about one hour precipitation was complete, and the nature of the precipitates formed was as indicated for the same salts in Table III.

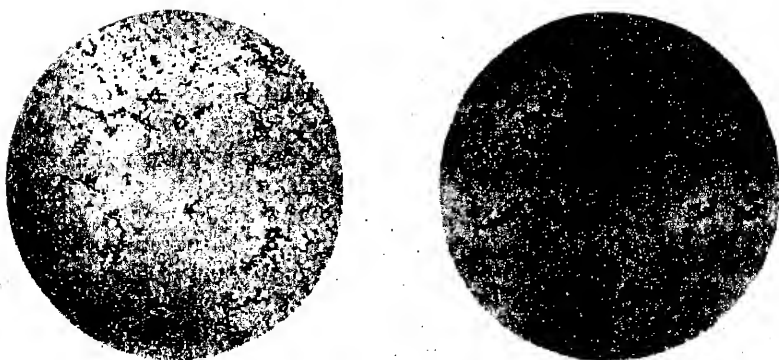
As a further experiment, precipitates were formed by passing the sol-forming gases into dilute solutions of LiCl, NaCl, and KCl previously saturated with sulfur dioxide. After the concentration of the salt had been reduced below the precipitation value through adsorption by the



(I) Precipitate formed with NaCl



(II) Precipitate formed with KCl



(III) Precipitate formed with BaCl₂

FIG. 1.—Macrographs of Sulfur Precipitates: Left, Immediately After Neutralizing Sol; Right, After Agitating to Agglomerate Coagulum.

precipitate, a sol was obtained in each case. However, the precipitate first formed was identical in character with that obtained by adding the electrolyte to the sol. It is therefore evident that, in this case, the nature of the precipitate is not determined by the degree of dispersity and percentage supersaturation at the beginning of the precipitation.

MICROSCOPIC OBSERVATIONS

Since the form of the precipitate is apparently due in the last analysis to the manner in which the individual colloidal particles come together after neutralization by the several electrolytes, attempts were made to view the process microscopically. As might be expected this was unsuccessful, since the colloidal particles are invisible in the ordinary microscope. Moreover, little information is obtainable from the microscopic observation of the precipitates formed with the different electrolytes. From the observations recorded in the preceding section, it seemed probable that the precipitates thrown down by lithium and sodium ions consist of aggregates of colloidal particles which have not coalesced, while the precipitates obtained with barium ions consist of masses formed by the coalescence of the individual particles. However, this difference obviously cannot be demonstrated with the ordinary microscope. Further observations were therefore made with the ultramicroscope.

ULTRAMICROSCOPIC OBSERVATIONS

Attempts were made to view the coagulation process in the presence of electrolytes by means of the Jentzsch (Leitz) and the Siedentopf cardioid²⁴ (Zeiss) ultramicroscopes. The results were not satisfactory with either apparatus. On account of the depth of the Jentzsch cell, the precipitated clumps settled out of the range of the objective as they formed and therefore could not be kept in focus. On the other hand, the cardioid cell is too shallow (depth of liquid under observation, 0.002 mm.) to allow the clumps to form, the individual neutralized particles merely settling out after the Brownian movement had ceased.

Since the process of clump formation was not readily followed, observations were made, by means of the cardioid ultramicroscope, of the nature and behavior of previously formed clumps. These observations were accomplished in the following way: About 1 cc. of sulfur sol was mixed with slightly more of the electrolyte than the amount necessary for complete precipitation and the mixture was allowed to stand for several minutes, after which a drop of the liquid containing

²⁴ For a theoretical discussion of the cardioid condenser, see Siedentopf, *Kolloidchem. Beihefte*, 23, 218 (1926).

a considerable amount of precipitate was placed in the ultramicroscope cell and examined. Differences in the ultimate structures of the precipitates are brought out in a striking way in the plates, Figure 2. The first row of ultramicroscopic pictures, Figure 2 (I), shows the precipitate obtained with lithium chloride, focussed at different levels in the clump. The individual particles are clearly discernible, separated by a film of water. In the second row of pictures, Figure 2 (II), is shown a portion of a large clump formed with potassium chloride, and Figure

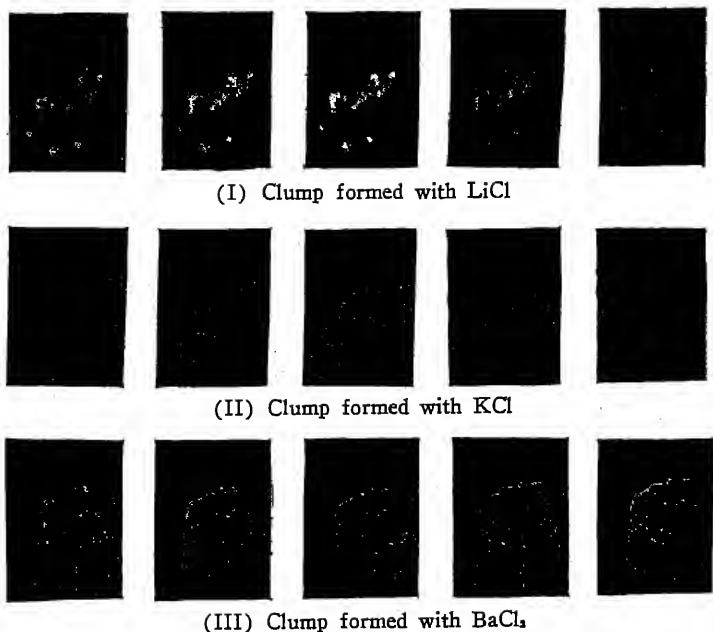


FIG. 2.—Ultramicroscopic Views of Clumps of Precipitated Sulfur Focused at Different Levels.

2 (III) shows a portion of a clump formed with barium chloride. In the last two cases it is clear that the individual particles have coalesced into a more or less uniform anhydrous clump of plastic sulfur. The smoothness of the edges of these two clumps shows particularly well the effect of the coalescence of the particles and the surface tension of the plastic mass.

Still more illuminating is the behavior of a hydrous clump formed in the presence of lithium or sodium ion, when treated with a solution containing the more readily adsorbed and less hydrated potassium, caesium or barium ion. The observations were made, as before, with

the cardioid ultramicroscope. In this apparatus the container for the sol is a quartz disc with a central depression 0.002 mm. deep and about 12 mm. in diameter, surrounded by a circular moat about 2 mm. deep and 5 mm. wide, into which any excess liquid is expelled when the quartz cover glass is put on. Two holes were bored on opposite sides of this cover glass in the same position as the moat, so that the latter could be cleaned out and refilled with any solution desired. The removal of liquid from the moat was accomplished by means of a narrow strip of filter paper rolled into a pointed rod, which was inserted into a hole in the cover glass and served as a wick. The moat was refilled by means of a medicine dropper drawn out to a capillary.

To make the observations, the cardioid cell was filled with a suspension of sulfur neutralized with lithium or sodium chloride. The moat was cleaned out and a satisfactory clump was brought into the field of view. The moat was then filled with a second solution containing the more strongly absorbed potassium, caesium, or barium ion, which diffused into the liquid bathing the precipitate and gradually, with repeated changing of the liquid in the moat, displaced the more weakly adsorbed sodium or lithium ion. This was accompanied by a marked change in the physical character of the clump, which change was followed photographically by means of an Ernemann motion picture camera especially constructed for microscopic work. The microscope used a glycerin-immersion objective of 3 mm. focal length (N. A. 0.85), and the ocular employed had a magnifying power of 20 diameters. The magnification is roughly estimated at 1,500 diameters. The light for the microscope was supplied by a 17.5-ampere carbon arc, a single quartz lens placed near the arc serving as a collimator. The beam of light was passed through a 2-inch thickness of 5 per cent copper sulfate solution and a 12-inch thickness of water in order to cool it. For the purpose of minimizing vibration it was found necessary to mount the camera and microscope on separate bases.

The motion pictures of the changes taking place in the precipitates were made by taking one exposure every two seconds. Projection at normal speed increases the apparent velocity of the change approximately thirty times. The photographs accompanying this paper were made from sections of the motion picture negative taken at suitable intervals to show the nature of the change. The phenomena observed under different conditions will be considered in order:

DEHYDRATION AND COALESCENCE OF PARTICLES

Experiment 1. The first observations were made on a clump thrown down by sodium chloride, in which the individual colloidal particles were

rather widely scattered but were imbedded in a sheath of adsorbed water. When the adsorbed sodium ion was replaced by barium ion in the manner described above, the excess water flowed out of the clump, leaving the colloidal particles stranded in approximately their original positions. At the same time there was an apparent contraction in the volume of the ultramicros. The photograph of the original clump, Figure 3 (I, *a*) discloses the individual particles, but the voluminous hydrous character of the clump gives it a somewhat hazy appearance since all portions could not be brought into focus at the same time. Figure 3 (I, *b*) shows the same clump after the excess water had flowed out of it, leaving the contracted particles.

Experiment 2. Since in the first experiment the particles were too widely scattered to come in contact after the excess adsorbed water was removed, attention was directed to a more compact clump obtained with sodium chloride, in which the ultramicros were somewhat unevenly distributed. In this case, replacing the sodium ion with barium ion produced the same phenomena described in Experiment 1; but, in addition, the particles in intimate contact flowed together or coalesced into three or four minute, slightly hydrous particles. This transformation is pictured in Figure 3 (I, *c, d, e*).

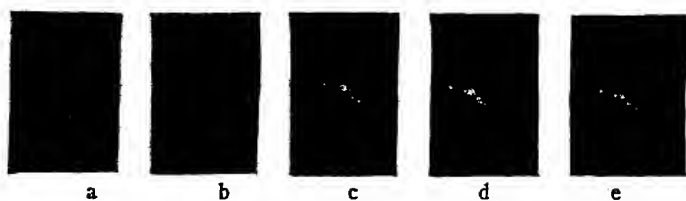
Experiment 3. Experiment 2 was repeated with a somewhat more uniform clump. In this case, the substitution of barium ion for sodium ion caused the entire gelatinous mass to coalesce into a single particle. See Figure 3 (II).

Experiment 4. On account of the more gelatinous character of the precipitate obtained with lithium chloride, ultramicroscopic observations were made on a relatively large gelatinous clump as barium ion replaced the lithium ion. In this case there was a very marked shrinkage in volume, all the particles coalescing into a small uniform ball. This change is illustrated in Figure 3 (III).

Observation of a projection of the motion picture film of which this plate is a sectional copy shows clearly the optical effects produced by the outflow of the adsorbed water. By visual observations in diffused light too dim to photograph, it is possible actually to see the convection currents in the water surrounding the clump. The striking character of this phenomenon can scarcely be imagined from the still pictures alone.

Experiment 5. Since potassium ion gives plastic sulfur, a clump formed with lithium ion was treated with potassium chloride. The loss of water and coalescence is illustrated in Figure 3 (IV).

Experiment 6. Since the precipitate formed with caesium ion is even more plastic than that formed with potassium, Experiment 5 was repeated, substituting caesium chloride for potassium with the results shown in Figure 3 (V).



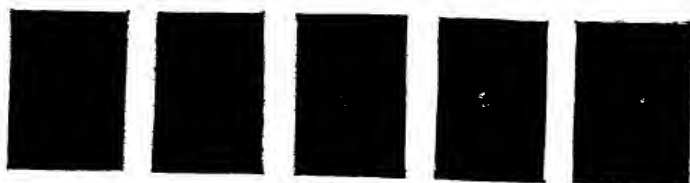
(I) Na displaced by Ba



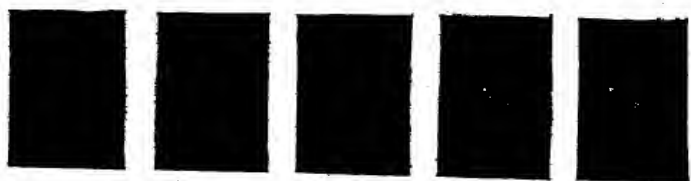
(II) Na displaced by Ba



(III) Li displaced by Ba



(IV) Li displaced by K



(V) Li displaced by Cs

FIG. 3.—Ultramicroscopic Views of the Behavior of Hydrous Sulfur Clumps on Displacing a Highly Hydrated Neutralizing Ion with a Less Hydrated Ion.

REVERSIBILITY OF PRECIPITATION

Attention has been called to the fact that the coagulum obtained with lithium or sodium ions is (1) gelatinous, (2) composed of the individual particles which have not coalesced and (3) readily reversible on washing out the precipitating ion. On the other hand, the precipitates obtained with potassium, caesium, and barium ions are (1) plastic, (2) formed by the coalescence of the ultramicros and (3) practically completely irreversible. It has been shown also that clumps do not form if the neutralization of the ultramicros is carried out in the shallow cardioid cell; instead, the individual particles settle out and remain at rest. This latter observation suggested experiments to show the factors which determine the reversibility of a precipitate. The method of procedure was as follows:

A monodisperse sol was placed in the cardioid cell, the excess sol was removed from the moat, which was filled with the solution containing the desired coagulating ion. After a sufficient amount of electrolyte had diffused into the cell to cause complete neutralization of the particles, the electrolyte was removed from the cell and pure water substituted. The electrolyte gradually diffused into the pure water, which was changed repeatedly.

Experiment 1. The sol was first neutralized below the critical value by sodium chloride. After removing the excess electrolyte, the Brownian movement was resumed by all the particles, showing that the neutralization was completely reversible.

Experiment 2. A dilute solution of barium chloride was used as the precipitating electrolyte. In spite of repeated changing of the water through a period of 48 hours, the particles showed no signs of again taking on the Brownian movement. This experiment demonstrates quite clearly that the neutralization process is irreversible even in the absence of coalescence if the adsorption of the precipitating ion is too strong.

Experiment 3. Since potassium and barium ions give a similar type of precipitate when the particles are allowed to coalesce and since the adsorption of the univalent potassium ion is much weaker than that of the bivalent barium ion, it seemed possible that the precipitation with potassium ion in the cardioid cell, where there is no coalescence, would be reversible. This proved to be the case, the neutralized particles taking on the Brownian movement in 30 to 40 minutes after the beginning of the washing to remove the potassium ion.

As we have seen, Freundlich²⁵ attributes the characteristic properties of Odén's sol to the presence of pentathionic acid in the micelles. He is therefore led to the conclusion that the reversibility of a sulfur

²⁵ *Kolloidchem. Beihefte*, 16, 234 (1922).

precipitate depends on the formation of a stable pentathionate with the precipitating ion. This view is not tenable, for in the case of potassium we have a precipitate which is reversible or irreversible depending on whether or not the particles are allowed to coalesce. Moreover, there appears to be no reason for believing that the lithium and sodium pentathionates should be any more stable than the corresponding potassium or barium salts. Certainly this is not true of the well known, closely related dithionates.

The results of the above observations indicate that precipitation is irreversible (1) when there is coalescence of particles to form relatively large clumps and (2) when the precipitating ion is so strongly adsorbed that it is not readily removed by washing, irrespective of whether or not there has been a coalescence of ultramicros.

THEORETICAL

From a survey of the observations recorded in the preceding section, it appears that the physical character of precipitated sulfur thrown down in the presence of alkali cations varies from gelatinous to plastic as we go down in the series from lithium to caesium. Likewise, the precipitate thrown down by alkaline earth cations changes in the same way as we go down in the series from magnesium to barium. In other words, precipitates formed in the presence of those ions which are generally recognized as the most highly hydrated, are the most gelatinous, and the precipitation is reversed by washing; while the precipitates thrown down in the presence of the less hydrated ions are dense and plastic, and the precipitation is not reversed by washing.

The order of hydration of the alkali cations is believed to be: $\text{Li} > \text{Na} > \text{K} > \text{Rb} > \text{Cs}$; and of the alkaline earth cations: $\text{Mg} > \text{Ca} > \text{Sr} > \text{Ba}$. Unfortunately, no one has yet succeeded in measuring quantitatively the ion hydration, much less its dependence on concentration, temperature, and the presence of foreign substances in the solution. Nevertheless, ion hydration numbers are frequently given. Thus, Remy²⁶ assumed that an ion moves like a sphere in a viscous liquid according to the well known Stokes formula, $u = 2/9 \cdot r^2 (d_1 - d_2) g / \eta$, where u is the velocity, r the radius and d_1 the density of the sphere, and d_2 and η the density and viscosity of the medium, respectively. The mobility of the ions calculated in this way is too large when the atomic radius is assumed to be the radius of the ion. From the greater radius which must be assumed to obtain agreement with the Stokes formula—an increase which is attributed to a sheath of water—it is calculated that the lithium ion is associated with more than 120 molecules of

²⁶ *Z. physik. Chem.*, 89, 467 (1915).

water; the sodium ion with more than 66; potassium ion, 16; rubidium ion, 14; and caesium ion, 13. Hydrogen is assumed to be anhydrous. The order of magnitude of the hydration numbers determined from transference experiments by Washburn and Millard²⁷ is quite different from the results of Remy. Thus, if chloride ion is assumed to be anhydrous the number of molecules of water on the several cations is calculated to be: lithium, 4.7; sodium, 2; potassium, 1.3; caesium, 0.7; and hydrogen, 0.3. If the chloride ion is assumed to have 4 molecules of water, the values for the cations become: lithium, 14; sodium, 8.4; potassium, 5.4; caesium, 4.7; and hydrogen, 1. Recent theories of strong electrolytes would indicate that hydrogen is relatively highly hydrated. Thus Bjerrum²⁸ calculates the hydration number of hydrogen to be 8 and that of potassium 0. Jablczynski and Wisniewski²⁹ conclude from freezing point measurements that lithium is combined with 11 molecules of water, sodium with 3 and potassium with 0. Schremer gives for hydrogen 10-11 and for lithium 6.5-7.5 molecules of water, respectively. On the assumption that hydrogen is monohydrated, Baborovsky³⁰ concludes that lithium holds 35 molecules of water, sodium 22 to 25 and potassium 6. Other investigators give still different values, but those recorded suffice to show that the absolute value for the degree of hydration of the ions under any specified conditions, is not known. All observers agree, however, that the hydration falls off in the alkali series from lithium to caesium and in the alkaline earth series from magnesium to barium. Born³¹ has shown further that the radius of the ions increases in the series from lithium to caesium, and Fajans³² and Born³³ have calculated the heats of hydration of the ions from the lattice energies of binary salts and have found them to decrease from lithium to caesium and from magnesium to barium. This merely confirms the order of hydration of ions in the two series, but gives no indication of the degree of hydration. Indeed, Fajans is of the opinion that the so-called hydration of ions does not connote the formation of ion hydrates of definite stoichiometric composition,³⁴ but rather that, due to the electrostatic charge of the ions, the oppositely charged part of the polar water molecule is turned toward the ion and this in its turn exerts an attractive force on the next molecule. This type of dielectric polarization would proceed continuously in the water surrounding the ion. From this point of view it would follow that the so-called hydration values will be relative numbers only. For a series of ions having the

²⁷ *J. Am. Chem. Soc.*, **37**, 694 (1915).

²⁸ *Z. anorg. Chem.*, **109**, 275 (1920).

²⁹ *Roczniki Chem.*, **1**, 116 (1921).

³⁰ *Chem. Listy*, **19**, 297 (1925).

³¹ *Z. Elektrochem.*, **26**, 401 (1920).

³² *Ber. physik. Ges.*, **21**, 401 (1920).

³³ *Z. Physik.*, **1**, 45 (1920).

³⁴ Cf. also Baborovsky and Velisek, *Chem. Listy*, **21**, 227 (1927).

same valence, the dielectric polarization will vary with the specific nature of the ion and with its size. In general, the concentration of the charge on a very small ion will exert a greater attractive force on the polar water molecules than the same charge on a larger ion.

Observations on the precipitation of sols with cations having the same valence, disclose that the adsorption of an ion, which determines its precipitating power, may be closely related to its degree of hydration.³⁵ If one accepts Fajans' view that the hydration consists in the formation of a polarized water envelope, the process being accompanied by a positive heat effect, one should expect the adsorption of an ion to be accompanied by a partial dehydration, the extent of which will be determined by the heat of hydration of the ions. Since we have seen that both the heat of hydration and the amount of hydration decrease in the alkali series from lithium to caesium and in the alkaline earth series from magnesium to barium, it follows that the adsorptive capacity and coagulative action should likewise decrease in the same direction, as the results show.³⁶

It is of interest to enquire into the probable thickness of the film of water surrounding the sulfur particles. Pettijohn³⁷ found the maximum thickness of a water film on some glass pearls made in Germany to be about 128μ . With another lot of glass of different origin the maximum was about 68μ . With river sand the estimated thickness varied from 285μ with 10-mesh sand to 114μ with 60-mesh sand. From observations by Barus³⁸ on the formation of fog, it is calculated that the thickness of the water film under the conditions of the experiments is 0.1 to 0.8μ when the nucleus has a diameter of 2.6μ and 0.05 to 0.5μ when the nucleus has a diameter of 3.6μ . With sugar charcoal, Bijl³⁹ was able to show that the Gibbs layer of water was more dense than the main bulk of the liquid. He calculated the thickness of this layer to be 0.68μ , which is below the limit of visibility with the ultramicroscope. Harkins and Ewing⁴⁰ found that when 10 grams of ignited charcoal was treated with 1 gram of water, the water was attracted onto the surface of the charcoal by an attractive force equivalent to 37,000 atmospheres, and that under this pressure, the water was compressed to 75 per cent of its original bulk. The high visibility of the film between the discharged sulfur particles would seem to indicate that the density of the adsorbed water is relatively high, the refractivity being of a similar order to that of sulfur. The visual observation of ultramicroscopic convection currents accompanying the dehydration,

³⁵ Cf. Lachs and Lachman, *Z. physik. Chem.*, 123, 303 (1926).

³⁶ Cf. Weiser, *J. Phys. Chem.*, 29, 955 (1923).

³⁷ *J. Am. Chem. Soc.*, 41, 477 (1919).

³⁸ *Phil. Mag.* (6), 4, 24, 262 (1902).

³⁹ *Rec. trav. chim.*, 46, 763 (1927).

⁴⁰ *J. Am. Chem. Soc.*, 43, 1787 (1921).

supports this view. In a relatively dense gelatinous mass of sulfur precipitate, the thickness of the film of water between the ultramicros appears to be, by comparison with the size of the ultramicros, about $50\mu\mu$.

In the light of these considerations it seems reasonable to conclude that when the sulfur particles are partly neutralized by the adsorption of highly hydrated ions, the particles retain an envelope of water, so that the coagulated mass is an agglomerate of ultramicroscopic particles which have not coalesced. The film of adsorbed water together with the water entrained during the agglomeration process gives a flexible hydrous mass, which is known as a gelatinous precipitate. The ultramicroscopic observations on gelatinous sulfur formed by coagulation of the sol with highly hydrated lithium, sodium, or magnesium ion gives definite visual confirmation of the nature of a gelatinous precipitate deduced by Weiser ⁴¹ several years ago.

Since the highly hydrated ions that yield gelatinous precipitates are not adsorbed strongly, and since the ultramicros retain their individuality in such precipitates, it follows that washing out the excess of precipitating ion should cause reprecipitation. This is very readily accomplished with the precipitates formed with the univalent lithium and sodium ions. Practically complete reprecipitation of precipitates formed in the presence of magnesium and zinc ions can also be effected, but the washing must be more thorough because of the stronger adsorption of the divalent ions.

Conditions are quite different if the neutralization of the particles below the critical value is accomplished by the adsorption of ions that are not sufficiently hydrated to maintain a film of water of sufficient thickness or rigidity to prevent coalescence of the individual ultramicros. This condition is realized with potassium, caesium, and barium ions. After neutralization, the ultramicros collide and coalesce, giving a more or less uniform mass of plastic sulfur which cannot be reprecipitated to give a sol, no matter how thoroughly the precipitate is washed. If the individual particles are prevented from coming together by precipitating in the shallow cardioid cell, reversal can be accomplished by washing if the relatively weakly adsorbed potassium ion has been used to effect neutralization, whereas if the more strongly adsorbed, bivalent barium ion is employed it cannot be displaced by washing and reversal is impossible.

The formation of sulfur precipitates in quantity, either by neutralizing the sol by the addition of electrolytes or by carrying on the reaction between sulfur dioxide and hydrogen sulfide in the presence of elec-

⁴¹ Cf. Bogue, "The Theory and Application of Colloidal Behavior," McGraw-Hill Book Co., New York, 1924, p. 387.

trolytes, can be visualized as follows: The first step following the formation of colloidal particles is their neutralization below the critical value necessary for agglomeration. When two or more such particles collide they either adhere or coalesce, the combination forming the nucleus for a larger clump. For the formation of a visible clump it is immaterial whether the particles actually coalesce or are held apart by a cushion of water. From the first collisions of discharged particles to form sub-microscopic or microscopic nuclei the general mechanism is the same. A larger clump enmeshes a smaller one, and is in turn enmeshed by a clump larger than itself. The entire process resembles the accumulation of driftwood in a swollen stream. The growth of a clump therefore may be regarded as autocatalytic in nature. The shapes and sizes of the ultimate clumps depend upon the number and manner of chance collisions, except that the weakness of the binding forces in the gelatinous precipitates makes impossible the formation of very large clumps in the absence of packing [cf. Fig. 1 (I)]. When the particles coalesce, the size of the clumps is limited only by the quantity of material available. The fundamental nature of the clumps, that is, whether they are flexible, gelatinous, and readily reprecipitated or are hard or plastic and non-precipitable, depends on whether or not the conditions are favorable for the coalescence of the ultramicros. This in turn depends on the nature and hydration of the adsorbed precipitating ion, in the manner above described.

SUMMARY

(1) A critical discussion of the von Weimarn Law of Corresponding States for the Precipitation Process has been given. It has been shown that the law in its simplest form is frequently inapplicable, and that, in certain cases, it may be of little value for predicting the form of a precipitate in advance of the precipitation experiments.

(2) Among the factors other than the percentage supersaturation which influence the physical character of precipitates are: (a) the specific tendency of the particles to adsorb the solvent; (b) the shape of the particles and (c) the effect of the adsorption of ions.

(3) The element sulfur furnishes a satisfactory substance for studying the specific influence of the adsorption of ions on the form of a precipitate, since the physical character of precipitated sulfur varies more or less continuously from gelatinous and reversible to plastic and completely non-reversible when thrown down in the presence of the lyotropic series of ions from lithium to caesium and from magnesium to barium.

(4) Neutralization of Odén's sulfur sol below the critical value with a highly hydrated, weakly adsorbed ion, such as lithium or sodium, gives

a gelatinous precipitate composed of the individual micelles separated by a film of adsorbed water. Such a precipitate is readily reprecipitated by washing out the neutralizing ion. Photographs are given showing the macroscopic and ultramicroscopic appearance of typical clumps.

(5) When the sulfur sol is neutralized with a slightly hydrated, strongly adsorbed ion, a plastic precipitate is obtained in which the individual particles have lost their identity due to coalescence. A precipitate of this type is not reversed by very thorough washing. Photographs are given showing the macroscopic and ultramicroscopic appearance of the clumps.

(6) Ultramicroscopic observation of the change taking place when a highly hydrated cation is removed from a gelatinous sulfur clump, by displacing with a less hydrated, more strongly adsorbed cation, shows a very marked shrinkage as the result of the loss of adsorbed water and the coalescence of the particles. Ultramicroscopic convection currents in the surrounding liquid, due to the outflow of the adsorbed water, are visible during the change. Motion pictures have been made of this change in the physical character of the clumps under the influence of various ions, and selected views from the motion pictures are included in this paper.

(7) When the sulfur sol is neutralized in the cell of the cardioid ultramicroscope, so that the neutralized particles do not collide with one another, the precipitation is reversible or irreversible depending on whether or not the adsorbed neutralizing ion may be removed by washing, and independently of whether the particles would coalesce if allowed to collide.

(8) It is concluded that: A reversible precipitate of any substance will be obtained when a sol is neutralized under such conditions as to prevent coalescence, either (a) by the intervention of a film of adsorbed solvent or (b) by preventing collisions of the neutralized particles. In either case it is essential that the adsorption of the neutralizing ion be sufficiently weak to permit its removal by washing.

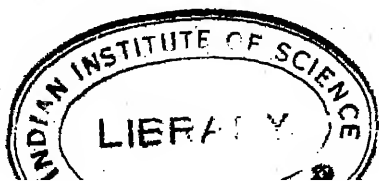
(9) The shapes and sizes of the individual precipitated clumps of sulfur depend upon the number and manner of chance collisions during the precipitation process.

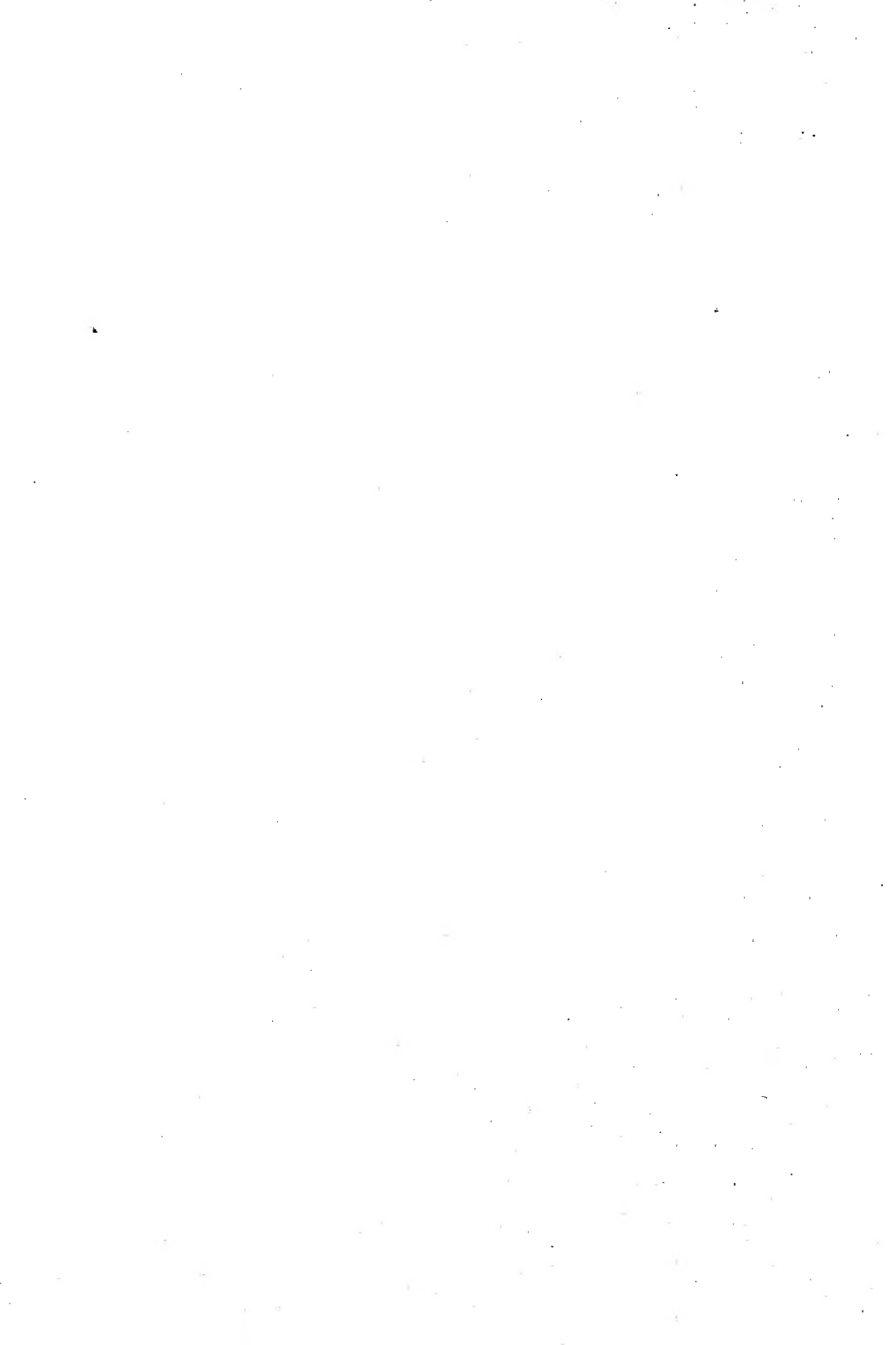
(10) The fundamental nature of the precipitated sulfur clumps depends upon whether or not the conditions under which the precipitation is carried out favor the coalescence of the ultramicros, which is in turn dependent upon the nature and degree of hydration of the adsorbed neutralizing ions.

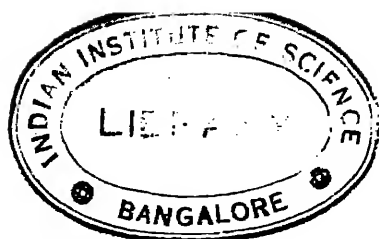
*The Rice Institute,
Houston, Texas.*

152

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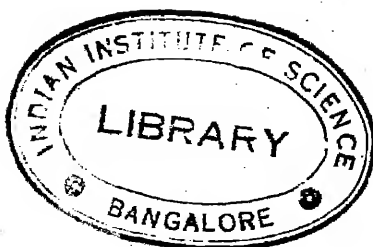


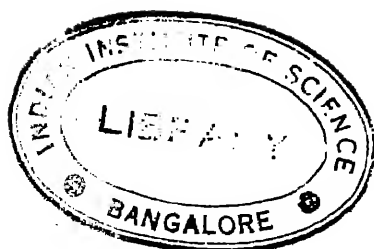


AUTHOR INDEX

- | | | |
|---|---|-----|
| Abramson, Harold A. | Cataphoresis of Blood Cells and Inert Particles in Sols and Gels and Its Biological Significance | 115 |
| Bancroft, Wilder D. and C. E. Barnett | The Adsorption of Methylene Blue by Lead Sulfate | 73 |
| Barnett, C. E. | See Bancroft and Barnett | 73 |
| Borsook, H. | See Wasteneys and Borsook | 155 |
| Briggs, David R. | Surface Conductance | 41 |
| Burton, E. F. and Mrs. Beatrice Reid Deacon | The Effect of Temperature on the Coagulation of Copper Colloidal Solutions | 77 |
| Cunningham, G. E. | See Weiser and Cunningham | 319 |
| Deacon, Mrs. Beatrice Reid | See Burton and Deacon | 77 |
| Fanselow, J. R. | The Influence of Electrolytes and Non-Electrolytes upon the Optical Activity and Relative Resistance to Shear of Gelatin Systems | 237 |
| Gallay, W. | See Whitby, McNally and Gallay | 225 |
| Giffen, F. J. | See Kenrick and Giffen | 53 |
| Gordon, Neil E. | See Krantz and Gordon | 173 |
| Hardy, Sir William B. | Living Matter | 7 |
| Harkins, William D. | Electrical Relations at Surfaces, the Spreading of Liquids, the Thickness of Surface Films and the Drop Weight and Ring Methods for the Determination of Surface Tension..... | 17 |
| Harrison, E. W. | See Laing, McBain and Harrison | 63 |
| Hastings, A. Baird | The Role of Hemoglobin in the Blood..... | 139 |
| Hauser, E. A., H. Miedel and M. Hünemörder | New Microscopic Methods in Connection with the Problem of Vulcanization | 207 |
| Holmes, Harry N. and Robert C. Williams | The Uniform Distribution of Catalysts Throughout Porous Solids | 283 |
| Hünemörder, M. | See Hauser, Miedel and Hünemörder | 207 |
| Jones, H. C. | See Neville and Jones | 309 |
| Kenrick, Frank B. and F. J. Giffen | The Effect of Adsorbed Water on the Electrical Conductivity of Powders | 53 |
| Krantz, John C. Jr., and Neil E. Gordon | Emulsions and the Effect of Hydrogen-Ion Concentration upon Their Stability | 173 |
| Laing, M. E., J. W. McBain, and E. W. Harrison | Adsorption of Sodium Oleate at the Air-Water Interface | 63 |
| Lambert, R. H. | See Sheppard and Lambert | 265 |
| Lucké, Balduin | See Mudd, Lucké, McCutcheon and Strumia.. | 131 |
| McBain, J. W. | See Laing, McBain and Harrison | 63 |
| McBain, J. W., W. F. K. Wynne-Jones and F. H. Pollard | The Activity and Adsorption of p-Toluidine in the Surface of Its Aqueous Solution..... | 57 |
| McCutcheon, Morton | See Mudd, Lucké, McCutcheon and Strumia.. | 131 |
| McNally, J. G. | See Whitby, McNally and Gallay | 225 |
| Miedel, H. | See Hauser, Miedel and Hünemörder | 207 |
| Moloney, P. J. and Edith M. Taylor | Fractionation of Diphtheria Antitoxic Plasmas | 109 |
| Mudd, Stuart, Balduin Lucké, Morton McCutcheon, and Max Strumia | Methods of Studying the Surfaces of Living Cells, with Especial Reference to the Relation between the Surface Properties and the Phagocytosis of Bacteria | 131 |

Neville, H. A. and H. C. Jones	The Study of Hydration Changes by a Volume-Change Method	309
Nichols, J. B.	The Development of the Ultracentrifuge and Its Field of Research	287
Olsen, Fred	Influence of Gel Structure Upon the Technology of Smokeless Powder Manufacture..	253
Pollard, F. H.	See McBain, Wynne-Jones and Pollard	57
Sheppard, S. E. and R. H. Lambert	Grain Growth in Silver Halide Precipitates..	265
Stamm, Alfred J.	The Structure of Softwoods as Revealed by Dynamic Physical Methods	83
Strumia, Max	See Mudd, Lucké, McCutcheon and Strumia..	131
Taylor, Edith M.	See Moloney and Taylor	109
Trumbull, H. L.	Preparation and Properties of Aqueous Rubber Dispersions	215
Wasteneys, H. and H. Borsook	The Effect of Emulsification in the Peptic Synthesis of Protein	155
Weiser, Harry B. and G. E. Cunningham	Adsorption of Ions and the Physical Character of Precipitates	319
Whitby, G. S., J. G. McNally and W. Gallay	Studies of Organophilic Colloids	225
Williams, Robert C.	See Holmes and Williams	283
Wynne-Jones, W. F. K.	See McBain, Wynne-Jones and Pollard.....	57





SUBJECT INDEX

Activity and Adsorption of p-Toluidine in the Surface of Its Aqueous Solution	57
Adsorbed Water, the effect of on the Electrical Conductivity of Powders...	53
Adsorption of Ions and the Physical Character of Precipitates	319
Adsorption of Methylene Blue by Lead Sulfate, the	73
Adsorption of Sodium Oleate at the Air-Water Interface	63
Adsorption of p-Toluidine in the Surface of Its Aqueous Solution, the Activity and	57
Blood, the Rôle of Hemoglobin in the.....	139
Blood Cells and Inert Particles in Sols and Gels and Its Biological Significance, Cataphoresis of	115
Catalysts, the Uniform Distribution throughout Porous Solids.....	283
Cataphoresis of Blood Cells and Inert Particles in Sols and Gels and Its Biological Significance	115
Cells, Methods of Studying the Surface of Living, with Especial Reference to the Relation between the Surface Properties and the Phagocytosis of Bacteria	131
Coagulation of Copper Colloidal Solution, the Effect of Temperature on the Colloids, Studies of Organophilic	77
Conductance, Surface	225
Conductivity of Powders, the Effect of Adsorbed Water on the Electrical....	41
Copper Colloidal Solution, the Effect of Temperature on the Coagulation of..	53
Diphtheria Antitoxic Plasmas, Fractionation of	77
Drop Weight Method for the Determination of Surface Tension	109
Electrical Relations at Surfaces	17
Emulsions and the Effect of Hydrogen-Ion Concentration Upon Their Stability	173
Emulsification in the Peptic Synthesis of Protein, the Effect of	155
Films, Surface, Thickness of	17
Gel Structure, Influence of upon the Technology of Smokeless Powder Manufacture	253
Gelatin Systems, the Influence of Electrolytes and Non-Electrolytes upon the Optical Activity and Relative Resistance to Shear of	237
Grain Growth in Silver Halide Precipitates	265
Hemoglobin in the Blood, the Rôle of.....	265
Hydration Changes by a Volume-Change Method, the Study of	309
Hydrogen-Ion Concentrations upon the Stability of Emulsions, the Effect of.	173
Interface, Adsorption of Sodium Oleate at the Air-Water	63
Ions and the Physical Character of Precipitates, Adsorption of.....	319
Lead Sulfate, the Adsorption of Methylene Blue by	73
Liquids, Spreading of	17
Living Matter	7

Matter, Living	7
Methylene Blue, the Adsorption of by Lead Sulfate.....	73
Microscopic Methods in Connection with the Problem of Vulcanization, the..	207
Optical Activity and Relative Resistance to Shear of Gelatin Systems, Influence of Electrolytes and Non-Electrolytes upon	237
Organophillic Colloids, Studies of	225
Phagocytosis of Bacteria, Methods of Studying the Surfaces of Living Cells, with Especial Reference to the Relation between the Surface Properties and the	131
Plasmas, Fractionation of Diphtheria Antitoxic	109
Porous Solids, the Uniform Distribution of Catalysts Throughout	283
Precipitates, Adsorption of Ions and the Physical Character of	319
Protein, the Effect of Emulsification in the Peptic Synthesis of	155
Ring Method for the Determination of Surface Tension	17
Rubber Dispersions, Preparation and Properties of Aqueous	215
Silver Halide, Precipitates, Grain Growth in.....	265
Smokeless Powder Manufacture, Influence of Gel Structure upon the Technology of	253
Sodium Oleate, Adsorption of at the Air-Water Interface	63
Softwood as Revealed by Dynamic Physical Methods, the Structure of.....	83
Spreading of Liquids	17
Structure of Softwoods as Revealed by Dynamic Physical Methods, the.....	83
Surface Conductance	41
Surface Films, Thickness of	17
Surface Tensions, Determination of, Drop Weight and Ring Methods for....	17
Surfaces, Electrical Relations of	17
Synthesis of Protein, the Effect of Emulsification in the Peptic	155
p-Toluidine in the Surface of Its Aqueous Solution, the Activity and Adsorption of	57
Ultra-Centrifuge and Its Field of Research, the Development of the.....	287
Vulcanization, New Microscopic Methods in Connection with the Problem of	207

